Kesterson Reservoir 1997 Biological Monitoring Report and 1998 Biological Monitoring Plan

Prepared for

US Bureau of Reclamation

Mid-Pacific Region

February 1998

CH2M HILL

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Section 1
Introduction

A meeting of participants from the U.S. Bureau of Reclamation (Reclamation), Central Valley Regional Water Quality Control Board (RWQCB), U.S. Fish and Wildlife Service (Service), and CH2M HILL occurred on September 11, 1995, to discuss the results of biological monitoring conducted at Kesterson Reservoir and how these results can be used to meet the objectives set forth by the RWQCB for closure of Kesterson.

At that time a decision was made to change the biological monitoring program from routine annual monitoring of the biota to smaller, focused studies concentrating on the biota or habitats at the highest risk that would be complemented by intermittent monitoring of selected biota. This report presents the results available to date for the studies conducted during 1997.

Overview

During the early to mid 1980s, elevated environmental selenium (Se) levels at Kesterson Reservoir (Merced County, California) were responsible for significant mortalities and embryo deformities in aquatic bird species (Ohlendorf et al., 1986; Ohlendorf, 1989). To alleviate the hazard of Se exposure to aquatic birds, studies and control actions were undertaken by Reclamation (USBR, 1987) who halted the inflow of drainage to Kesterson in June 1986. However, contaminants that had accumulated in the soil and biota remained, and in 1988 Reclamation dewatered the reservoir and filled all areas so they were above average winter groundwater levels. This eliminated all permanent wetlands and reduced the formation of ephemeral pools, effectively changing Kesterson Reservoir from an aquatic to a terrestrial ecosystem (Ohlendorf and Santolo, 1994).

A biological monitoring program was implemented in 1987 to document habitat and faunal changes and Se concentrations among selected plants and animals. Biological monitoring at Kesterson was conducted annually through 1995. In 1996, an intermittent monitoring plan

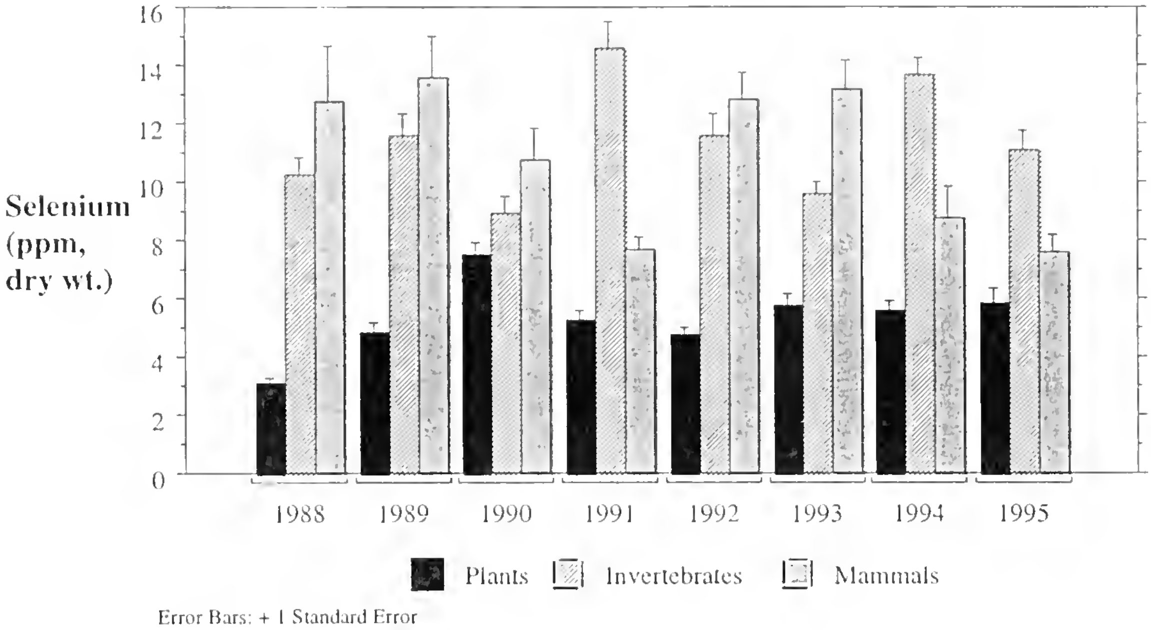
and focused studies were initiated. The general objectives of biological monitoring at Kesterson have been to:

- Assess the risks to local and migratory animals posed by Se levels in soil and biota at Kesterson Reservoir
- Provide a basis for adjusting Kesterson Reservoir management
- Verify the effectiveness of cleanup actions at Kesterson Reservoir
- Provide a basis for modifying future biological monitoring

The monitoring program has provided information necessary for management decisions by documenting existing ecological conditions and identifying trends in these conditions. The results of the biological monitoring efforts from 1987 through 1996 have been presented in annual reports (USBR, 1987 to 1996).

Since 1988, Se concentrations in sampled plants, invertebrates, and mammals have varied from one year to another. About five to ten percent of the Se in soil at Kesterson is in a soluble form, although this varies greatly (Wahl et al., 1993), and no trend of increasing or decreasing Se has developed in biota sampled from Kesterson (Figure 1-1). This variation may be attributed to environmental changes such as rainfall, timing of sampling, and habitat succession. Sampling results and predicted soil-water Se concentrations were used to model future Se concentrations in selected biota at Kesterson (CH2M HILL, 1993; Ohlendorf and Santolo, 1994). The results from the model predicted that Se concentrations would remain stable within the ranges described in Figure 1-1. Although Se concentrations in biota at Kesterson continue to be above reference levels, there has been no evidence of seleno-toxic effects in the terrestrial ecosystem.

Figure 1-1. Mean Selenium Concentrations in Plants, Invertebrates, and Mammals Collected from Kesterson Reservoir, 1988 to 1995



The monitoring program has provided general assessments regarding the status and trends of Se in biota at Kesterson, and it has included evaluation of adverse effects (although none have been found since 1988). To ascertain more specific cause-and-effect information and to better address the general objectives of the biological monitoring program, additional special studies were undertaken. The monitoring program was used as a framework to organize and focus those studies.

The 1996 and 1997 monitoring program focused on the areas that pose a higher risk or new conditions to wildlife at Kesterson, such as perennial vegetation, detritus, and Se inventory in the food web. Studies are being conducted to determine the effects of Se on upper trophic level consumers and small mammals. Monitoring efforts similar to the biological monitoring that has been conducted since 1987 will be conducted every 3 years (Table 1-1). The monitoring program will be adjusted based on the results of the additional studies. In this

way, data will be consistent and it will be possible to compare results to past conditions and identify trends.

Table 1-1. Intermittent Biological Monitoring and Focused Study Plan

Activity	1996	1997	1998	1999	2000	2001
Vegetation Surveys	-	-	X	-	-	X
Vegetation/Invertebrate/Soil Sampling	-	-	X	-	-	X
Small Mammal Sampling	-a	-	X	-	-	X
Nocturnal Surveys	-	-	X	-	-	X
Detritus and Mushroom Sampling	X	X	X	-	-	X
Bird Population Surveys	X	X	X	X	X	X
Bird Nesting and Reproduction	X	X	X	-	-	X
Ephemeral Pool Monitoring	X	X	X	X	X	X
Captive Kestrel Study	X	-	-	-	-	-
Wild Bird Sampling	X[b]	X	X	-[e]	-[e]	X
Nest Box and Telemetry[b, c] Study	X[c]	X[c]	X	-	-	X
Shrike Sampling and Banding	X[cd]	X[d]	X[d]	-[e]	-[e]	X[d]
Body Condition	X[b]	-	-	-	-	-

[a]Selected small mammals were collected for body condition studies.

[b]Selected wild birds trapped were used for body condition studies.

[c]The telemetry portion of the study will begin during the second year of the study (1997).

[d]The banding and blood sampling portions of the study will be conducted along with other bird trapping .

[e]Only banding will be conducted.

The 1997 monitoring and some focused studies have been completed; other studies are still underway. Results of the second year of the detritus and mushroom sampling, ephemeral pool monitoring, the nest box study, and the bird nesting and reproductive study are presented in this report. Captive kestrel studies and body condition studies have been completed and reports on the results of those studies have been submitted in separate reports. The bird population surveys, wild bird sampling, and the loggerhead shrike sampling and banding studies are currently being conducted and will be reported separately.

Studies

Biological monitoring of Se concentrations at Kesterson Reservoir has documented elevated levels throughout the food web. These levels have been compared to reference levels and, because data for terrestrial wildlife are limited, to results in aquatic system studies. Plants, invertebrates, mammals, and birds sampled generally are above suggested threshold levels for concern (3.0 to 8.0 ppm[1]) and many are above the suggested toxic threshold for adverse effects (8.0 ppm or greater). Although numerous samples are at levels expected to cause chronic or reproductive toxicity in birds and mammals, no effects have been observed during biological monitoring since the reservoir was filled. A series of laboratory and field studies was undertaken to further determine if there are no adverse Se effects to wildlife using Kesterson Reservoir. These studies, which are briefly described below, focus on providing information for interpreting the results of the past 9 years of biological monitoring at Kesterson.

Field Studies

Detritus and Mushroom Sampling

Sampling of plant litter, mushrooms, and sowbugs has identified the detritus pathway as one of the highest potential exposure pathways at Kesterson in terms of elevated Se concentrations. Monitoring of this system is being conducted to characterize the exposure risks associated with feeding on detritus and mushrooms. Organisms that feed primarily on detritus, such as sowbugs and mushrooms, have higher Se concentrations than herbivores, omnivores, and carnivores that have been analyzed. These detritivores are expected to continue to have elevated Se concentrations. To better characterize the detritus pathway, samples of detritus were collected during 1996 and 1997 from the 54 sampling stations located throughout Kesterson and analyzed for Se. The results are presented in Section 2. Se concentrations remain elevated in detritus, but overall concentrations were significantly lower than those found in detritus samples collected in 1988. Detritus from the Open habitat types (unfilled

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[1] All Se concentrations are reported on a dry weight basis.

areas where cattails were cut and left to decompose) had significantly higher Se concentrations than either Filled or Grassland sites. In additon, detritus from Open habitats collected in 1996 and 1997 was not statistically different from Open habitat samples collected in 1988. Although mushrooms were not abundant at Kesterson during 1997, those that were collected had significantly lower Se concentrations than mushrooms analyzed in other years.

Ephemeral Pool Sampling

Ephemeral, rainwater pools at Kesterson Reservoir that persist for greater than approximately one month during the winter season have been observed to harbor abundant populations of algae, aquatic insects, and crustaceans. The aquatic invertebrates are potential prey items for the abundant shorebirds in the area and the fauna of these small ponds has been sampled since 1992 with the intent of monitoring the Se contamination of these dietary items. The invertebrate prey items of the pools are of particular concern because the pools form and persist at the time the shorebirds are feeding and beginning to nest in the area. Water and dietary Se levels in Kesterson ephemeral pools in past years and in 1997 exceed risk thresholds for waterbird hatchability and teratogenesis (Ohlendorf and Santolo, 1994).

In February and March 1997, eight ephemeral pools were sampled for water and aquatic invertebrates. A total of six insect and six crustacean samples were obtained; all were analyzed for whole body tissue Se as composites of many hundreds of individuals per sample. The results indicated some of the highest values of bioaccumulation in aquatic organisms observed since Kesterson Reservoir was dewatered and filled in 1988. As in previous years, crustaceans (ostracods and cladocerans) averaged over twice the tissue Se concentrations of aquatic insects (water boatmen, beetles, and midge larvae). The results indicate a potential continuing source of significant Se exposure from prey items for shorebirds feeding in the Kesterson Reservoir area.

Bird Population Surveys

Bird populations fluctuate from year to year depending on a number of factors including climate, food abundance, and habitat changes caused by natural (e.g., plant succession) or

management activities. Bird population surveys have been conducted since 1989 as average daily bird use estimates by recording systematic observations at Kesterson several times per month and summarizing the data. This survey is used to identify population trends, changes of species using the reservoir, and seasonal changes in bird abundance and diversity. This information helps to time and interpret other studies (i.e., wild bird sampling and bird nesting and reproduction studies). In addition to the daily use surveys, belt transect surveys are also conducted during the spring and fall to estimate numbers of birds using Kesterson that are not counted by daily use survey techniques (e.g., songbirds such as sparrows, larks, wrens, and warblers).

Bird Nesting and Reproduction

The objectives of this study are to determine Se concentrations in eggs and to assess the reproductive success of birds nesting at Kesterson Reservoir. Reproductive success was evaluated by determining the frequencies of embryonic mortality and developmental abnormalities, as well as the hatching and fledging success of birds nesting during spring and summer of 1997.

The number and types of nests found at Kesterson reflect the change in use of the reservoir by various bird species. Typical of many grassland communities, there is relatively low species richness of nesting birds at Kesterson. Grassland bird communities in some areas tend to have low species richness partially due to periodic climate extremes that prevent drought-susceptible species from perennial settlement (Zimmerman, 1992). Many ground-nesting birds prefer open grasslands for nesting; the tall weeds and grasses that are found growing over much of Kesterson may exclude or limit nesting by these birds in some areas. Killdeer are such ground-nesting birds that prefer open areas for nesting, and primarily nest on the gravel roads around the perimeter of the Reservoir. In recent years, most nests were found along the east side of Kesterson, along the San Luis Drain. The high vegetation that grows up to the roads in some locations may limit the favorable nesting areas for killdeer. Low growing or possibly delayed growth of plants in some areas in the interior parts of Kesterson and along the west-side perimeter roads appeared to favor some nesting by killdeer in those areas in 1997 and higher numbers of killdeer nests were found than in recent years.

Other birds such as barn swallows seem to be nest-site limited and are not rebuilding old nests that have fallen or been destroyed by rain. (Swallows require a vertical surface and open water for nesting; those at Kesterson use vertical surfaces in the culverts along Gun Club Road.) As has been observed in recent years at Kesterson, barn swallows continue to nest at lower density.

No waterfowl, shorebird (other than killdeer), or raptors (other than kestrels and barn owls using nest boxes) successfully fledged young at Kesterson in 1997. Kesterson seems to provide only marginal nesting habitat for these species. No shorebird (other than killdeer) nests were found and waterfowl nests were lost to predation or abandonment. Two raptor nests on power poles were abandoned before they were completed. Most raptors are limited by the lack of nest sites (e.g., trees) at Kesterson and the available nest sites that were used (i.e., power poles) appear to lack features that provide stability for the nest (e.g., branches).

Nests and eggs are monitored closely at Kesterson and few unhatched eggs have been found in recent years. Although Se-related effects on bird reproduction at Kesterson have not been found since the filling operation, many eggs continue to have Se levels above median background levels of 1.9 ppm and above the 4.5 to 5.0 ppm threshold for above background avian contamination in nonmarine environments (Skorupa and Ohlendorf, 1991; J.P. Skorupa, U.S. Fish and Wildlife Service); six killdeer eggs were above the suggested avian threshold for reproductive impairment of 10 ppm (Heinz, 1996) and above the 14 ppm threshold egg Se level for stilt teratogenesis. Using a best-case scenario, two killdeer eggs collected in 1997 were above the threshold level for embryo teratogenesis for tolerant taxa (Skorupa et al., 1996). Thus, the potential for Se-induced reproductive problems continues to exist and in those clutches with egg Se levels above 14 ppm may be likely.

Few nests, other than killdeer nests, were found on Kesterson Reservoir in 1997. Overall, Se levels in the eggs collected in 1997 were similar to levels found in samples collected in 1991 and 1996 and significantly lower than eggs collected in all other years since 1988 (after filling), and no effects that could be attributed to Se toxicosis were observed. Still, bird eggs with elevated Se concentrations continue to be found at Kesterson, and some eggs (i.e., some killdeer eggs) have levels high enough to cause Se toxicosis in embryos.

The results of the nesting and reproductive studies conducted at Kesterson in 1997 are presented in detail in Section 4. Nests of mallard, American kestrel, killdeer, barn owl, lesser nighthawk, barn swallow, and European starling were sampled and monitored. Mallard, red-tailed hawk, and some killdeer nests were unsuccessful due to predation, abandonment, management activities, and high winds. No evidence was found implicating Se as the cause of nest failures. Se concentrations were generally similar or somewhat below concentrations in eggs from other years.

Wild Bird Sampling

Field monitoring of raptors and other birds utilizing Kesterson has been carried out for about three years and is continuing in order to provide data on exposure levels in these birds. Blood Se levels are measured in trapped birds, and birds are banded with U.S. Fish and Wildlife Service bands to provide further data if they are recovered at a later date. The bands identify birds using the site and may provide information on individual birds such as their survival, where they nest, and if they return to Kesterson. This information can be used to determine the effects to migrating birds and local populations. Also, birds trapped and banded in the fall and winter that are retrapped in the spring will provide information on resident birds that nest in the area and forage at the site. Species such as great horned owls and red-tailed hawks that begin nesting as early as February and March, and barn owls that may nest all year in the Central Valley (weather permitting), are specifically targeted in the winter to determine if they are at risk for reproductive impacts. With laboratory data described previously, blood levels in wild birds can be used to estimate their dietary and potential egg levels of Se (because many are sampled outside the nesting period). Species, seasonal, and life-stage (adult vs. immature) differences observed in blood Se levels will assist in characterization of, and indicate management alternatives for minimizing, Se risks to birds at Kesterson. Since beginning this effort in 1994, 187 predatory birds have been sampled for blood-Se concentrations (Table 1-2).

Table 1-2. Blood-Se Concentrations (ppm, dry wt.) in Predatory Birds at Kesterson, 1994 to 1998

Species	*n*	Range	Geometric Mean
Loggerhead shrike	14	4.9 - 20	11
American kestrel	38	2.0 - 12	3.9
Barn owl	82	1.5 - 12	4.3
Great horned owl	2	6.1 - 9.8	7.7
Burrowing owl	1	--	11
Northern harrier	6	6.3 - 15	9.4
Red-tailed hawk	44	2.1 - 8.7	3.9

This is an ongoing study that may be used for long-term monitoring at Kesterson. Samples are collected year-round; however, fall and winter are when trapping is most intensive. Identification of individuals using Kesterson is conducted all year, both opportunistically and during systematic bird surveys.

Shrike Sampling and Banding

The loggerhead shrike is a resident bird in the Kesterson area and is federally identified as a sensitive species. Monitoring efforts at Kesterson have indicated that these predatory passerine birds accumulate higher concentrations of Se in their tissues compared with raptor species studied at Kesterson. From January 1, 1996 to January 10, 1998, nine shrikes trapped on Kesterson have been color banded. These shrikes have a geometric mean blood Se concentration of 13 ppm ($n = 9$; Range = 9.8 - 20 ppm). They appeared to be in good condition (based on subjective evaluation of breast muscle and feather condition). Shrikes are primarily insectivorous and the levels reflect Se concentrations that often are higher in invertebrate samples than small mammal samples collected at the site (USBR, 1987 to 1996; Figure 1-1). Higher concentrations in shrikes may also be partially due to their having

relatively smaller home ranges and, consequently, increased foraging time on Kesterson. If so, loggerhead shrikes may represent a current "worst case scenario" with respect to dietary Se exposure at Kesterson, and may be at relatively higher risk of adverse reproductive effects than bird species that forage off-site to a larger extent. Given these considerations, more intensive study of this species' feeding behavior and Se exposure at Kesterson is being conducted. Shrikes are often trapped opportunistically while trapping other species. When shrikes are captured, they are sampled (blood) for Se analysis, and fitted with U.S. Fish and Wildlife Service bands and colored leg bands for subsequent identification.

Colored leg bands help provide information on the range of shrikes, habitat types used within Kesteson, and the survivorship of individual shrikes. Information is also being collected on seasonal use of Kesterson by shrikes and foraging patterns during reproduction. This information is collected during other ongoing activities, and sampling, banding, and observations are incorporated into other studies and carried out with minimal cost and effort. Color banding may also help to correlate the blood Se levels in shrikes with areas within the reservoir and specific food chain items. The results of wild bird sampling and loggerhead shrike banding and sampling will be presented in a separate report.

Nest Box and Telemetry Study

Prior avian monitoring at Kesterson indicates that birds nesting at the site do not exhibit impaired reproduction. However, it is unknown whether this is due to Se ingestion being relatively low because birds forage partially off-site, selective foraging on dietary items low in Se, and/or reduced sensitivity to Se compared to aquatic species. In order to address some of these important data gaps, artificial nest boxes were placed on and around the site during the late winter-early spring period of 1996 to attract breeding birds, which could then be studied closely for signs of exposure to and effects of high dietary Se concentrations. For purposes of comparison with the laboratory feeding study, and because of known occurrence and utilization of Kesterson, the target species in this study is the American kestrel. However, other cavity-nesting bird species occupying the nest boxes also provide useful data in this study. The results of the nest box study for 1997 are presented in Section 5 of this report. In 1996, most nest boxes were occupied by European starlings. In 1997, starling eggs

and nesting material were removed from nest boxes when they were found. Eggs, blood, and livers were collected for Se analysis. A limited number of food items being fed to the nestling starlings were also collected. Se concentrations varied among nest boxes by the general location and possibly by individual foraging range. No Se-induced effects were observed and nest success was high in all nests where egg clutches were initiated.

Three off-site and five on-site nest boxes were occupied by kestrels in 1997. All of the off-site and three of the on-site nests were successful. Both unsuccessful nests were abandoned after the female died or disappeared prior to egg laying. Greater occupancy by kestrels is expected in future years based on field observations at Kesterson and success of other nest-box studies.

In 1997, once nest boxes were occupied by kestrels, foraging ranges of breeding birds were studied using radiotelemetry to monitor bird movements. This allowed determination of the extent to which the birds forage at Kesterson and estimation of dietary Se exposure, both of which can be compared with the reproductive success of these birds. Pellet analyses for American kestrels provide an approximate prey composition for this species and improved dietary Se estimations. Blood sampling of breeding birds and offspring for Se analyses, together with correlation data from the laboratory feeding study, were used to further characterize exposure patterns.

One barn owl nest box was placed on Kesterson in 1996 and occupied in both 1996 and 1997. The female adult owl had been trapped, banded and sampled (blood) in July 1995, before the nest box was erected. Blood Se concentrations have been collected over three years during the reproductive period from this bird (July 1995, May 1996, and May 1997) and from her nestlings for two years. Three of the four eggs laid by this pair hatched and fledged successfully (one of the four eggs was collected for Se analysis).

Body Condition

One effect of Se toxicity in birds and mammals is wasting of muscle tissue. Weight, however, is a poor indicator in wild animals because of variability in size, reproductive condition, sex, and measurement accuracy. Body composition analysis was conducted to

provide information on the condition and health of wildlife at Kesterson. This analysis was conducted noninvasively for selected bird and mammal species using total body electrical conductivity (TOBEC). Lean body mass, percent body fat, and total body water were compared to reference and laboratory measurements to determine if effects of Se not observed by gross examination and body measurements can be detected using this method. Body composition was also measured in captive kestrels.

The conclusions reached from captive and field studies were that:

- The body condition of kestrels and potentially other wildlife that consistently forage at Kesterson may be affected by Se concentrations currently found in dietary items.
- The body condition effects of Se observed in captive birds are likely to be more pronounced in wild birds that are periodically faced with food scarcity and other stresses (e.g., migration, temperature extremes, competition).
- Although direct embryotoxicity may not necessarily be observed in high Se areas, reduced body condition may be an indirect mechanism by which Se can impair reproduction. Furthermore, due to apparent persistent effects, birds having wintered in high Se areas may not have adequate resources to either survive migration or breed successfully in subsequent months.
- Reduced body condition in parent birds may affect the provisioning of chicks.
- Due to the high natural variability typically observed in wild animals, the utility of using EM-SCAN for detecting subtle Se-induced changes in condition is limited under field conditions. Other methods of body condition assessment may be effective for field diagnosis of Se effects.

Laboratory Studies

Captive Kestrel Study

Given the relatively limited area of Kesterson and low populations of nesting terrestrial birds available for analysis, it has been difficult to determine whether terrestrial species are experiencing impaired breeding as was found previously in aquatic birds. In addition, while several laboratory studies on the effects of Se on reproduction in waterfowl species have been carried out, such information on terrestrial birds is limited. Reproductive effects of Se on terrestrial birds may differ substantially from aquatic species, in part due to species differences and/or differences in Se availability in terrestrial versus aquatic environments. Hence, previously established dietary thresholds for acute, chronic, and reproductive toxicity, which are based on data from waterfowl and other aquatic species, may not be useful levels for the management of terrestrial populations. These studies were undertaken to: 1) determine whether Se levels found at Kesterson are likely to reduce reproductive success in a model terrestrial bird fed environmental levels of Se found at Kesterson; and 2) test existing dietary thresholds for applicability to this or similar species. Overall, the results of these studies have allowed more meaningful interpretation of field residue data and reduced uncertainty in predictions made regarding exposure of birds to Se at Kesterson and subsequent reproductive risks.

In 1994, male-female pairs of kestrels were maintained for 11 weeks on diets containing 5 or 9 ppm selenium (dry weight) as seleno-L-methionine, or naturally-incorporated selenium in the form of mammals collected at Kesterson Reservoir. Selenium concentrations in blood and excreta of male and female kestrels within groups were similar. Near-maximal mean selenium concentrations in blood were observed after the fifth week of treatment in the seleno-L-methionine treated kestrels and an approximately 1:1 ratio was observed between maximal blood concentrations and dietary concentrations. All treatment groups exhibited reduction of selenium concentration in excreta, but not in blood, to baseline values four weeks after treatment ended. No birds were observed to exhibit signs of general illness or selenium toxicity during the study.

In 1995, male-female pairs of kestrels were fed diets containing approximately 6 ppm (Low-Se) or 12 ppm (High-Se) selenium (dry weight) as selenomethionine for 11 weeks. Near-maximal mean selenium concentrations in blood were observed by Day 35 of treatment in the selenomethionine-treated kestrels. An approximately 1:1 ratio was observed between maximal blood concentrations and dietary concentrations and 1.7 - 2.8:1 between egg and diet concentrations. There were no significant changes in body weights of male kestrels during the study period compared with baseline weights, with one exception in the High-Se selenium group. Although there was poor reproductive performance in all groups, selenomethionine treated birds from the High-Se group appeared to have lower fertility. Low hatchability in all groups suggest non-treatment factors were responsible. No gross abnormalities were found in kestrels fed High-Se diets, although eggs contained about 25 ppm selenium. This suggests that kestrels, as well as several other bird species studied to date, are less sensitive than mallards to reproductive effects of selenium.

The main conclusions of this study were: 1) American kestrels receiving dietary selenium levels similar to levels expected in birds foraging 100 percent of their time on the reservoir (i.e., 12 ppm) had reduced fertility and reduced body condition (based on body fat and lean mass) when compared to Control kestrels, 2) American kestrels receiving dietary selenium levels similar to those levels found in their potential foods at Kesterson Reservoir (i.e., foraging about 50 percent of their time on the reservoir; 6 ppm) had similar reproduction to Control kestrels, and 3) this finding is consistent with the results of the ecological risk assessment conducted in 1993 (Ohlendorf and Santolo, 1994) that modeled the exposure risk to red-tailed hawks and northern harriers among other species found at Kesterson. They reported that Kesterson represents only a small portion of the range of most predators and Se levels in these and many other terrestrial species are not expected to accumulate to biologically significant levels. This study and monitoring at Kesterson Reservoir since 1988 suggest that at the current selenium levels found in prey items at Kesterson, most raptors are not at significant risk for population-level effects. However, individual birds utilizing Kesterson for foraging and nesting may have greater exposure to dietary selenium and, therefore, may be at risk.

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Section 2
Detritus and Mushroom Sampling

Introduction

Results of sampling conducted since 1988 identified the detritus pathway of plant litter, mushrooms, and sowbugs as one of the highest potential risk pathways at Kesterson in terms of elevated Se. This pathway is important for the movement of biologically incorporated Se because more plant biomass dies and is shed as litter than is consumed alive (Swift et al., 1979). For this reason, more bioavailable Se may reside in decomposers and in fresh or partially decomposed organic matter than in live plants or their consumers. Se, similar to other nutrients and trace elements such as nitrogen, phosphorus, and calcium, is recycled within the ecosystem. Plants and animals associated with detritus may obtain Se as it cycles through the system and may also acquire additional Se moving up from below the root zone. Decomposing organic matter below the surface at Kesterson may also contribute to Se in decomposers such as mushrooms and soil microbes.

Detritus samples were collected in 1988, soon after Kesterson Reservoir was dewatered and filled. That year, samples were collected only from Grassland and Open habitats because no detritus layer had yet formed on the newly filled areas. Detritus samples were again collected in 1996 and 1997.

Objective

The objective of the detritus and mushroom sampling program was to determine the Se accumulation in mushrooms and in the detritus layer at Kesterson and compare these results with those from other years.

Methods

Detritus

Detritus samples were collected during September at each of 54 sample stations by separating the organic layer of material from the mineral soils. This was accomplished by removing the loose top layer of material (i.e., loose plant material and other debris) by hand before the soil sample was taken. Each sample consisted of a composite of three samples collected within a radius of about 24 ft from the station's center. Samples were analyzed by Laboratory for Environmental Testing, Inc. (LET) for total Se.

Mushrooms

Mushroom samples were collected from Kesterson in all seasons and locations when they were found incidental to other activities and also during systematic surveys conducted in January and February. Mushrooms were identified as either Agaricus (*Agaricus* sp. are a common, non-poisonous meadow mushroom abundant at Kesterson from 1990 to 1994) or non-Agaricus. Agaricus are organic decomposers and heavy metal accumulators (Kovaks,1992). Samples for analysis were collected by removing sections of the gill and cap from three or more fruiting bodies (when available). The cap and gill sections of the mushroom were used because earlier studies had shown that the highest Se levels were found in this part of the fruiting body (USBR, 1992). The remainder of the fruiting body was removed and frozen for future disposal to limit further propagation of mushrooms at Kesterson. In 1994, Agaricus and non-Agaricus mushrooms were collected and compared and non-Agaricus mushrooms were compared in 1994, 1996, and 1997.

Results

Detritus

Geometric mean Se concentrations in detritus samples collected in 1997 are compared in Table 2-1. Mean Se concentrations in detritus samples collected from Open habitats were

usually over ten times higher than those in samples from the other habitats. Within habitat types, Se concentrations in Filled habitat were significantly lower in Trisection 3 than in Trisections 1 and 2. Se concentrations in detritus collected from Open habitats in Trisection 1 were significantly higher than in detritus collected in Trisection 3 Open habitats.

Table 2-1. Se Concentrations (ppm, dry wt.) in Detritus Samples, 1997

	Trisection 1		Trisection 2		Trisection 3		Kesterson Total	
Habitat Type	**GM**	**[*n*] (Range)**	**GM**	**[*n*] (Range)**	**GM**	**[*n*] (Range)**	**GM**	**[*n*] (Range)**
Grassland	8.3 Aa	[5](3.6 - 24)	4.6 Aa	[6](1.4 - 12)	3.5 Aa	[6](1.7 - 11)	5.1 A	[17](1.4 - 24)
Filled	9.3 Aa	[5](4.9 - 19)	6.2 Aa	[6](3.9 - 14)	1.9 Ab	[6](0.89 - 6.9)	4.5 A	[17](0.89 - 19)
Open	104 Ba	[5](22 - 340)	82 Bab	[6](40 - 200)	28 Bb	[6](15 - 37)	63 B	[17](15 - 340)
Kesterson Total	**19 a**	**[15](3.6 - 340)**	**13 ab**	**[18](1.4 - 200)**	**5.7 b**	**[18](0.89-37)**	**11**	**[51](0.89 - 340)**

Note: Geometric mean concentrations within columns sharing the same capital letter are not significantly different (p≤ 0.05). Geometric mean concentrations within rows sharing the same lower case letters are not significantly different (*P*≤ 0.05)

Mushrooms

Se concentrations in mushrooms for 1997 are compared by trisection and habitat type in Table 2-2. Fewer mushrooms have been found growing at Kesterson since 1994 and, of those found, there were no Agaricus. Too few samples were collected this year to determine if differences were significant among trisections or habitats. Thus, no significant differences were observed in mushroom samples collected from Filled and Grassland habitats (trisections combined) or among the three trisections (habitats combined).

Table 2-2. Se Concentrations (ppm, dry wt.) in Mushroom Samples, 1997

	Trisection 1		Trisection 2		Trisection 3		Kesterson Total	
Habitat Type	**GM**	**[*n*] (Range)**	**GM**	**[*n*] (Range)**	**GM**	**[*n*] (Range)**	**GM**	**[*n*] (Range)**
Grassland	16	[1]		-	7.3	[2] (5.7 -9.3)	9.5	[3] (5.7 - 16)
Filled	8.3	[1]	18	[2] (3.8 - 89)		-	14	[3] (3.8 - 89)
Kesterson Total	**12**	**[2] (8.3 - 16)**	**18**	**[2] (3.8 - 89)**	**7.3**	**[2] (5.7 - 9.3)**	**12**	**[6] (3.8 - 89)**

In 1994, Se concentrations from Agaricus mushrooms were compared to concentrations from non-Agaricus species. Se concentrations in the Agaricus were significantly higher than non-Agaricus mushroom samples (Table 2-3). Non-Agaricus mushrooms collected in 1994 had significantly higher Se concentrations than non-Agaricus mushrooms collected in 1996 and 1997.

Table 2-3. Comparison of Se Concentrations (ppm, dry wt.) in Agaricus to Non-agaricus Mushroom Species collected in 1994 and 1997

Species	*n*	Range	Geometric Mean Se
Agaricus - 1994	8	420 - 1100	686A
Non-Agaricus - 1994	11	61 - 944	294B
Non-Agaricus - 1996	6	3.8 - 89	12C
Non-Agaricus - 1997	12	2.6 - 360	13C

Note: Geometric mean concentrations not sharing the same capital letter are significantly different ($P \leq 0.0011$).

Discussion

Se concentrations in detritus have been significantly reduced in from 1988 to 1997 in Open habitats from 140 ppm to 54 ppm, respectively (Table 2-4) and in Grassland and Filled habitats from 55 ppm to 5.2 ppm, respectively at Kesterson (Table 2-4; Grassland and Filled Habitats were not significantly different between Grassland and Filled habitats in 1996 and 1997 and were combined within each year as "Other"). In Open habitats, where a large amount of detritus was left on the soil surface by cutting and disking cattails and other vegetation, Se concentrations are lower than 1988 samples, but continue to be significantly higher than Se concentrations in Grassland and Filled habitats. However, Open habitat covers less than either Filled or Grassland habitats (Figure 2-1) and vegetation in these habitats is becoming more similar as succession continues (Ohlendorf and Santolo, 1994; USBR, 1995). Also, Kesterson is a relatively small area (about 517 ha) in a larger mosaic of uncontaminated grasslands and wetlands (Figure 2-1). Therefore, wildlife feeding at Kesterson are likely to use Open habitats to a lesser extent than Filled and Grassland habitats and, depending on home range of a species, are likely to forage off-site to a great extent.

133230 RP ZZ Kesterson Map B&W2 2-11-98sbm

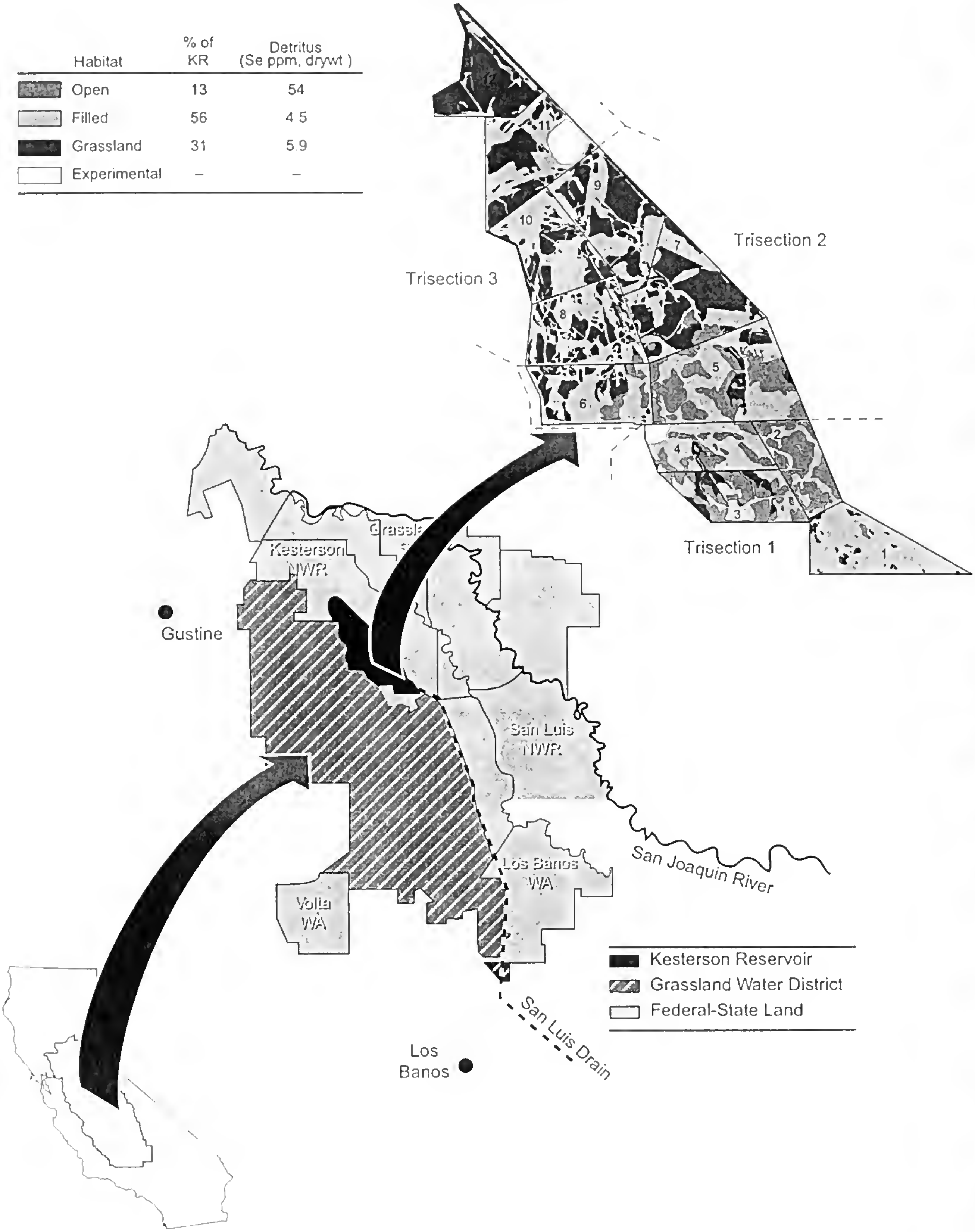

Figure 2-1
Kesterson Reservoir Location and Habitats

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Table 2-4. Se Concentrations (ppm, dry wt.) in Detritus Samples, 1988 - 1997

Year - Habitat	*n*	Range	Geometric Mean Se	Notes
1988 - Open	9	67 - 317	140 A	
1988 - Other	33	8.0 - 170	55 a	No detritus from Filled habitat
1996 - Open	18	7.1 - 340	66 BC	
1996 - Other	36	1.0 - 22	4.8 b	No difference between Filled and Grassland
1997 - Open	18	9.2 - 340	54 C	
1997 - Other	36	0.89 - 130	5.2 b	No difference between Filled and Grassland

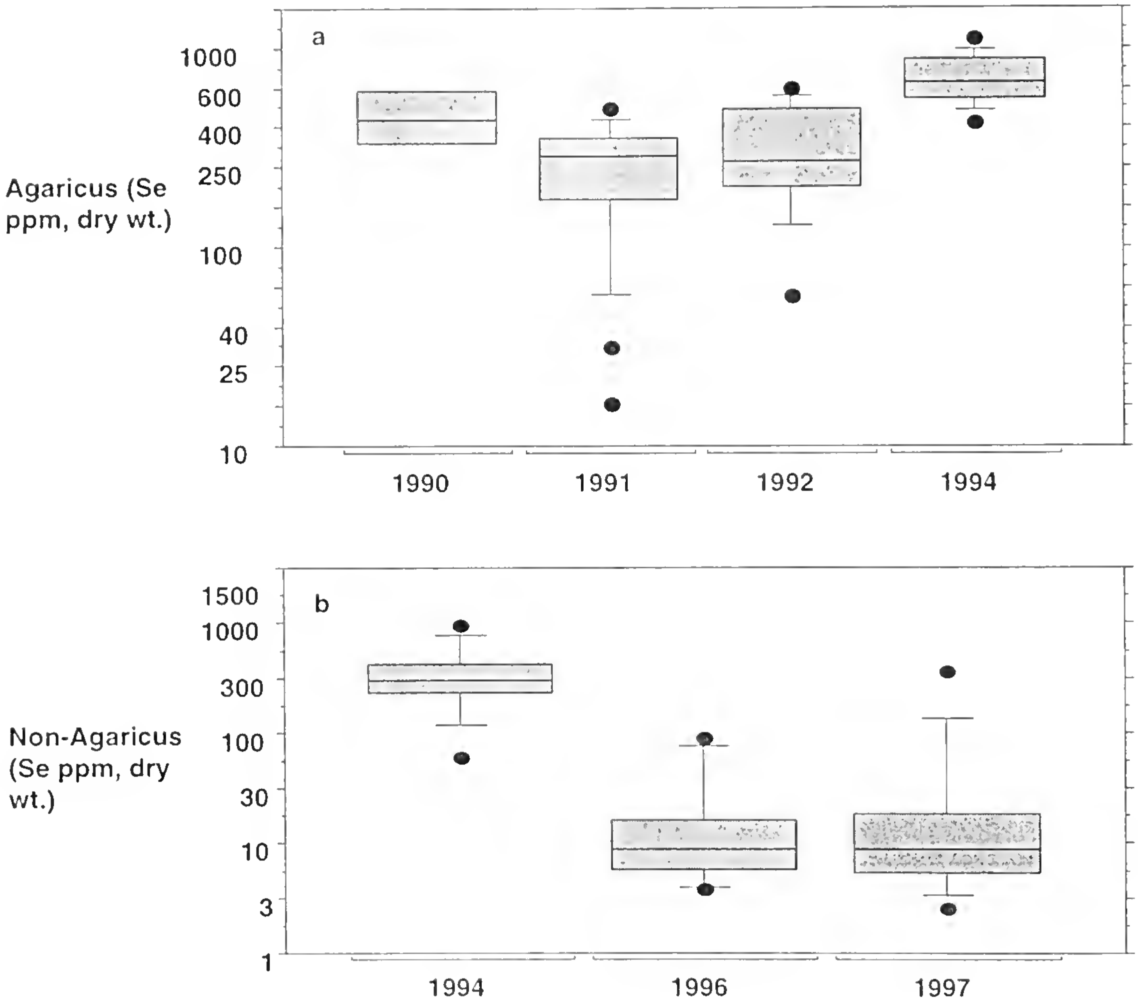
Note. Geometric mean concentrations within Open habitats not sharing the same capital letter are significantly different ($P \leq 0.05$)
Geometric mean concentrations within Other habitats not sharing the same lower case letter are significantly different ($P \leq 0.05$).

From 1991 to 1994 mushrooms were abundant in areas of sparse vegetation throughout Kesterson. Of these mushrooms, Agaricus were the most common species found. Since then, mushrooms have not been abundant at Kesterson. Many factors (such as plant cover, soil moisture, and temperature) affect the growth of these fungi, and soil moisture conditions may also affect uptake of Se by mushrooms (Swift et al., 1979).

Agaricus mushrooms had elevated levels of Se in all years that they were found at Kesterson and showed no declining trend from 1990 to 1994 (Figure 2-1a). It is unknown if wildlife at Kesterson feed on Agaricus preferentially over other mushroom species, but it is likely that wildlife feed on non-poisonous available mushrooms regardless of species. Therefore, mushroom species were pooled to evaluate the changes and potential risk from Se in mushrooms found at Kesterson. Of the mushrooms found at Kesterson, Se concentrations were significantly lower in 1996 and 1997 than in 1994 when non-Agaricus mushrooms were sampled (Figure 2-1b; Table 2-3). However, It is as yet too soon to determine if Agaricus will become abundant once again if conditions at Kesterson change and, if they are found again, whether they will have elevated Se concentrations similar to those found from 1990 to 1994. Other mushroom species may accumulate Se similar to levels found in Agaricus in past years. However, at this time Se concentrations in mushrooms (i.e., all species found) are lower than concentrations from other years (Table 2-5). Whether this is an anomaly due to

climatic conditions, edaphic factors, species of mushrooms, or a small sample size is not known.

Figure 2-2. Selenium Concentrations in Mushrooms Collected from Kesterson Reservoir, 1990 to 1997



Since filling, the detritus and decomposers have contained the highest levels of Se among plants and animals characterized for potential risk to wildlife at Kesterson Reservoir. However, exposure of wildlife to Se along this pathway may be low because of limited feeding on detritus or mushrooms. Concentrations in mushrooms and detritus (i.e., overall concentrations) were lower in 1997 than in previous years sampled and fewer mushrooms were found growing on Kesterson, further reducing the risk of Se exposure through this

pathway. Sowbugs, which have not been collected since 1995, will be collected in 1998 along with detritus and mushrooms. This will allow a reevaluation of the risk posed by the detritus pathway.

Although Se concentrations in both detritus and mushrooms are significantly lower in almost all samples in the terrestrial food pathway, it is too early to determine if this is the beginning of a trend or simply a fluctuation due to some unique conditions or events. Continued monitoring to track Se concentrations in the various components of the detritus system will provide information to further characterize the potential risk of this system to wildlife using Kesterson.

Table 2-5. Se Concentrations (ppm, dry wt.) in Mushroom Samples, 1988 - 1997

Year	*n*	Range	Geometric Mean Se	Notes
1990	4	320 - 660	448 A	Many mushrooms observed
1991	22	16 - 501	211 A	Many mushrooms observed
1992	13	56 - 630	285 A	Many mushrooms observed
1994	25	61 - 1100	475 A	Many mushrooms observed
1996	6	3.8 - 89	12 B	Few mushrooms found
1997	6	2.6 - 360	13 B	Few mushrooms found

Note: Geometric mean concentrations not sharing the same capital letter are significantly different ($P \leq 0.05$).

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Section 3
Ephemeral Pool Sampling

Introduction

Rainwater pools form at a number of locations in Kesterson Reservoir during the winter rainy season. Ponding has been observed in most winters and those pools surviving for greater than approximately one month have been observed to develop populations of aquatic invertebrates. Se salts dissolve in the pools from the underlying soil and there is a concern that waterfowl or shorebirds feeding and nesting at Kesterson when ephemeral pools are present in the late winter/ early spring may accumulate significant amounts of Se from their aquatic invertebrate prey in the pools Ohlendorf and Santolo, 1994).

Aquatic insect and crustacean species have been sampled from Kesterson ephemeral pools each winter since 1992 except for 1994, when pools did not persist long enough to develop significant invertebrate populations. Annual monitoring of water and invertebrates in the ephemeral pools that are most persistent has been conducted as a means of tracking potential exposure of aquatic birds to selenium (through their diet).

Methods

Persistent rainwater pools were sampled at four locations in Kesterson Reservoir on February 7 and eight locations on March 27 and 28, 1997; locations are shown in Figure 3-1. All of the pools had persisted for at least a month and had developed noticeable invertebrate populations. Samples were collected in the following order:

1. Raw, unfiltered water was sampled from just below the surface of the pools, with care not to collect resuspended sediment. A single sample was collected per pool.

2. Invertebrates were collected by kick nets and aquarium nets from the water column and the surface layer of the sediment. One to three samples were collected per pool, depend

ing on availability of each species and the relative ease of separation of single species from mixed species assemblages, pool detritus, and filamentous algae.

The invertebrates were placed from nets into clean plastic pans, hand picked by forceps, and placed into clean Whirlpak bags for storage and shipment to the laboratory. Microcrustacean zooplankton (*Daphnia* sp. and ostracods) were placed directly into Whirlpak bags from the nets. Each invertebrate sample consisted of many hundreds of individuals field-identified as probably being the same species. The samples were chilled in the field, frozen within a day, and shipped frozen to the analytical laboratory (LET) for analysis of total Se from whole-body, composite samples.

Results and Discussion

Results are shown in Table 3-1. Tissue Se concentrations ranged from 6.7 (in corixids) to 110 ppm (in ostracods/*Daphnia* mix). Waterborne concentrations ranged from 7.4 to 70 ppb total Se.

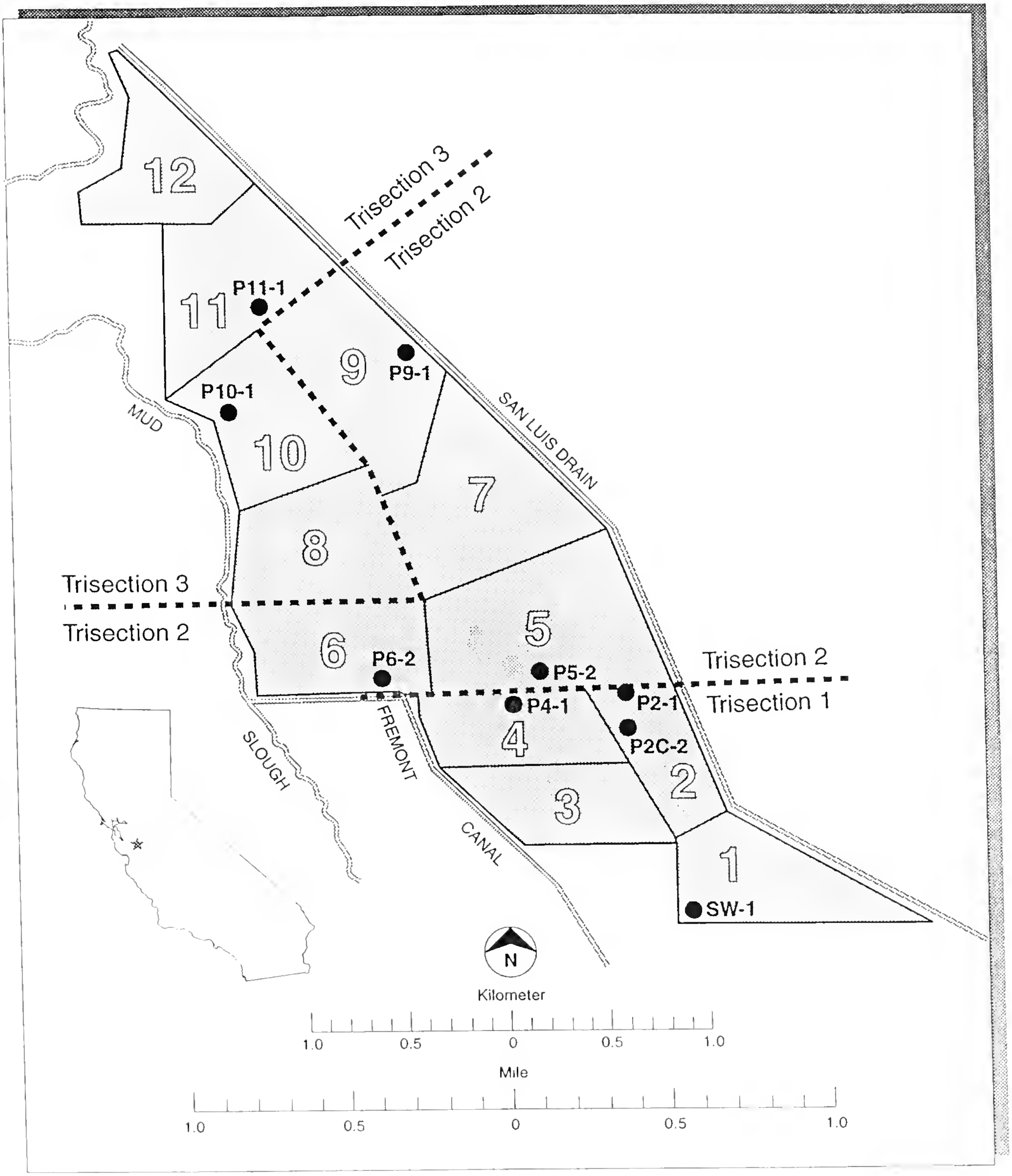

Figure 3-1
Kesterson Reservoir Ephemeral Pools Monitoring Sites
February 7, March 27, and March 28, 1997

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Table 3-1. Selenium Concentrations in Aquatic Invertebrates, Algae, and Water Collected from Ephemeral Pools at Kesterson Reservoir, 1997

(Collected in March, unless indicated otherwise)

Pool	Invertebrates	Se Concentration Tissue (ppm, dry wt.)	Se Concentration Water (ppb)
Pond 1			
SW-1	Midge larvae	61	
SW-1	Ostracod/*Daphnia* mix	48	
SW-1	**Pool Mean[1]**	**54.1**	**24**
Pond 2			
P2C--2	Filamentous Algae	26	
P2C--2	Midge larvae	48	
P2C--2	Ostracod/*Daphnia* mix	31	
P2C-2	**Pool Mean[1]**	**33.8**	39
P2-1	Filamentous Algae	60	
P2-1	Ostracod/*Daphnia* mix, February, 1997	86	
P2-1	Ostracod/*Daphnia* mix	110	
P2-1	**Pool Mean[1]**	**82.8**	**70**
Pond 4			
P4-1	Ostracod/*Daphnia* mix, February, 1997	9.1	-
Pond 5			
P5-2	Filamentous Algae	32	
P5-2	Beetle larvae	16	
P5-2	Corixid	23	
P5-2	Ostracod/*Daphnia* mix, February, 1997	65	
P5-2	Ostracod/*Daphnia* mix	39	

P5-2	**Pool Mean[1]**	**31.3**	**7.4**
Pond 6			
P6-2	Corixid	16	
P6-2	Ostracod/*Daphnia* mix, February, 1997	12	
P6-2	Ostracod/*Daphnia* mix	16	
P6-2	**Pool Mean[1]**	**14.5**	**16**
Pond 9			
P9-1	Corixid	21	
P9-1	Ostracod/*Daphnia* mix	30	
P9-1	**Pool Mean[1]**	**25.1**	**29**
Pond 10			
P10-1	Ostracod/*Daphnia* mix	48	
P10-1	Ostracod/*Daphnia* mix, duplicate	43	
P10-1	**Pool Mean[1]**	**45.4**	**30**
Pond 11			
P11-1	Corixid	6.7	15
All	**Insect Mean[1]**	**21.9**	
All	**Crustacean Mean[1]**	**35.4**	
Overall Means[1]		**30.5**	**23.8**

[1]"Means" are geometric means for invertebrates and single samples of water per pool.

Ephemeral pool water has been sampled in previous years from pools where invertebrates were collected. The 1992 through 1997 data indicate a positive and statistically significant correlation between the selenium concentration in water and aquatic invertebrate tissue. For all Kesterson Reservoir rainwater pool data since 1992, the two significant relationships are:

Insects: Log tissue selenium (ppm DW) =

0.66 + 0.45 Log water selenium (ppb) ($P<0.01$, $r^2 = 0.31$)

Crustaceans: Log tissue selenium (ppm DW) =

0.87 + 0.53 Log water selenium (ppb) ($P<0.01$, $r^2 = 0.32$.

The low r^2 values of the relationships may be explained by lags between changing pool water concentrations and invertebrate bioaccumulation and by the variable link between pool water and invertebrate diet item concentrations. Se concentrations for the most common invertebrate taxa in 1997 are shown in relation to samples taken from all previous years in Table 3-2. Samples collected in 1983 to 1988 were from the original reservoir prior to drying; samples since 1992 were collected from ephemeral pools that formed on the dried, partially filled reservoir.

Table 3-2. Average Tissue Se Concentrations (ppm, dry wt.) in Aquatic Invertebrates from Kesterson Reservoir, 1983 - 1997

	Aquatic Insects				Crustaceans			
	Corixids		Midge larvae		*Daphnia* sp.		Ostracods	
Year	GM	*n*	GM	*n*	GM	*n*	GM	*n*
1983	16 - 295[2]	-	200	1				
1984	19[1]	3	102[2]	3				
1987	13	13	89	24	31	1	31	1
1988	10	55	39	33				
1992[1]	11	4	6.7	2	18	4	36	1
1993[1]	6.8	9	9.1	2	15	8		
1995[1]	13	3	14	1	28	1		
1996[1]	17	4			57	2	60	1
1997[1]	15	4	54	2	35	12		

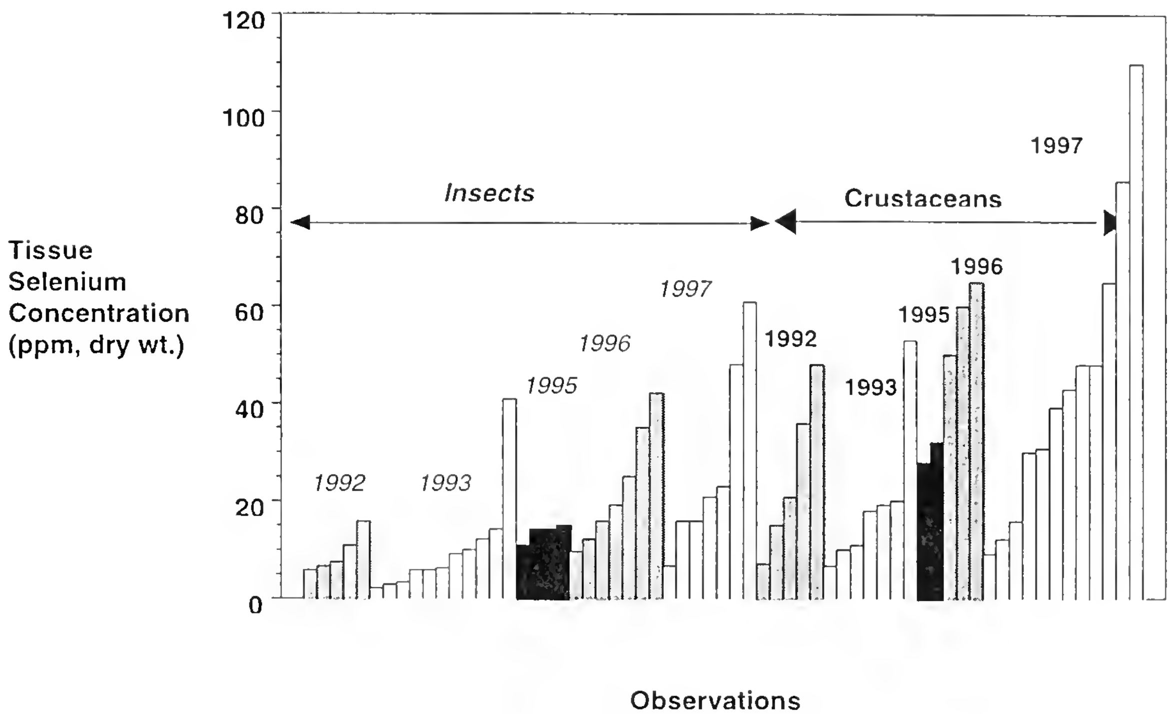
[1]Ephemeral pool samples; those prior to 1992 were from Kesterson ponds prior to dewatering and filling in 1988.

[2] from Saiki (1985), results as "insects" or "net plankton"

[2]from Schuler (1987), all diptera larvae

Midge larvae often have higher Se concentrations than crustaceans (Table 3-2), but overall, crustacean Se concentrations have consistently exceeded those of aquatic insects in recent years (Figure 3-2). The average selenium bioaccumulation factors (tissue Se concentration divided by waterborne Se concentration) for rainwater pool samples since 1992 at Kesterson are 1535 for insects and 2904 for crustaceans.

Figure 3-2. Aquatic Insect and Crustacean Tissue Selenium Concentrations from Kesterson Reservoir Rainwater Pools, 1992 to 1997



Analysis of variance results for insect and crustacean tissue Se results over time indicate a significant difference due to taxonomic category, time, and for the year/taxa interaction ($P < 0.05$, log-transformed data). The most significant change over time is caused by the increase in crustacean Se in 1996 and 1997 samples, although insects also have increased in geometric mean Se concentration during recent years (Table 3-2, Figure 3-2).

The greatest increase in rainwater pool invertebrate selenium concentrations over time has been observed from pools in Trisection 1. Ponds 1 and 2 were the historical area of drainwater influent to Kesterson and Trisection 1 had the highest sediment selenium

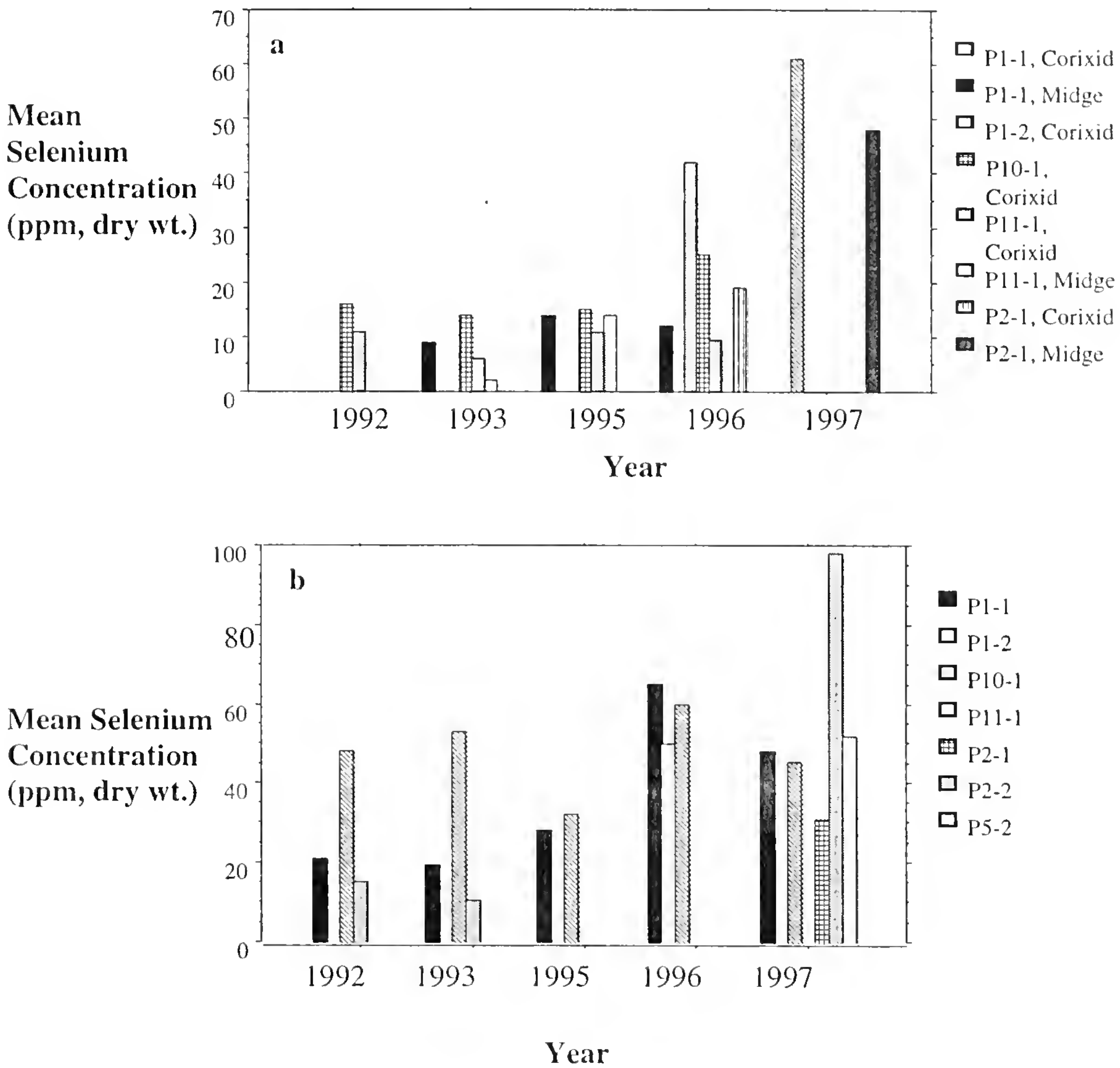
contamination even though fill was placed over much of the original sediments(see Figure 3-1).

Much of the apparent increase in bioaccumulation in 1997 is probably due to an increased sampling effort that resulted in collections from previously unsampled pools, increased numbers of midge samples, and increased numbers of samples from Trisection 1 as compared to previous years. Sediment-dwelling midges have always had the highest selenium concentrations of the Kesterson ephemeral pool insects (e.g., Tables 3-1, 3-2). The two high values for insects from 1997 shown in Figure 3-2 are both midge samples taken from pools in Ponds 1 and 2 (both in Trisection 1) (Table 3-1). Insects sampled in previous years (since 1988) were either not midges, not from Trisection 1, or both (the only exceptions are the highest insect value for 1993 [midge sample fom Pond 3] and the highest insect sample for 1996 [midges from Pond 1], Figure 3-2).

The crustacean data are also affected by sampling location. Taxonomic differences were less important. The two highest values for 1997 were from *Dapnia* and ostracods collected from the same pool near Gun Club Road in Pond 2. That pool had not been sampled previously and thus did not contribute to the longer term record. Similarly, the third highest crustacean value for 1997 was collected from a pool in Pond 5 that was previously unsampled.

Thus, the high values and "apparent trends" over time as shown in Figure 3-2 are all heavily influenced by sampling location and species sampled rather than change over time. Figure 3-3 shows tissue concentration values for the most common insect and crustacean taxa sampled at the most frequently sampled locations. Note that although midges tend to have greater concentrations than corixids, location of the sample also is important, and the highest corixid sample was found from pool P1-2 in 1996. The three highest concentrations are all from Trisection 1 samples (Figure 3-3a). Future investigations should resample pools for previously sampled taxa from previously sampled locations to better track trends at Kesterson. The possibility that Trisection 1 filled areas are experiencing increased selenium "wicking" through the fill material over time may be investigated by pairing water and invertebrate sampling with groundwater and soil investigations of those areas.

Figure 3-3. Selenium Concentrations in Invertebrate Taxa from Selected Ephemeral Pools, Kesterson Reservoir, 1992 to 1997. (a) Aquatic Insects (b) Micro-crustaceans (all as either *Daphnia*, ostracods, or both mixed).



Conclusions

Ephemeral rainwater pools remain a potential threat of exposure for Se toxicity to shorebirds and waterfowl feeding and nesting at Kesterson Reservoir. The concentrations of total Se in the tissues of aquatic invertebrates exceed toxicity thresholds for adverse hatchability and

teratogenic effects for birds potentially feeding on those items (Skorupa and Ohlendorf, 1991) and exceed background invertebrate values for comparable taxa at San Joaquin Valley reference sites (Skorupa et al., 1996). Waterborne concentrations observed in 1996 also exceeded the potential toxicity thresholds for foodchain bioaccumulation and exposure to birds. (Exposure would be mainly through feeding on the resident invertebrates of the pools.) All invertebrate samples collected in 1997 had Se at levels above the threshold for potential adverse effects on reproductive success (i.e., > 6.0 ppm DW; Heinz, 1996) .

The Se concentrations of ephemeral pool crustaceans generally exceeded those of aquatic insects (Table 3-2, Figure 3-2). However, it should be noted that sediment-dwelling midge larvae are often high in Se concentration (Table 3-1). Although there is a general, positive relationship between water Se and the tissue Se concentration of the pool's invertebrate inhabitants (see Results and Discussion, above), the relationships only explain about one third of the variance in tissue concentrations and therefore indicate there are significant unknown factors that influence aquatic invertebrate Se bioaccumulation. It is apparent that some aspect of life history, diet, or physiology of Se sequestration separates insects and crustaceans (as discussed by Skorupa and Ohlendorf, 1991). The groups sampled as part of this study are primarily herbivorous or detritivorous. The only exceptions are the beetle larvae and larvae of some midge species, which may be predatory.

The approximately doubled mean tissue Se concentrations of crustaceans compared to insects is probably due, at least in part, to physiological differences. Crabs analyzed for tissue bioaccumulation of selenium in San Francisco Bay showed highest tissue concentrations in the hepatopancreas (Saiki, 1985), a digestive organ of crustaceans not found in insects. The basic pattern in aquatic arthropod Se bioaccumulation results is reflected in the terrestrial arthropods at Kesterson, as well. Terrestrial crustaceans (sowbugs) consistently accumulate higher concentrations of Se than do terrestrial insects (USBR, 1995; Ohlendorf and Santolo, 1994).

It is also possible that a diet at least partially composed of detritus (e.g., as is likely for sowbugs or aquatic micro-crustacean grazers such as ostracods and *Daphnia*) leads to greater exposure or availability of dietary Se than might otherwise exist through either strictly

herbivorous or predatory pathways (which may be the case for most terrestrial and aquatic insects). Dietary pathways of aquatic or terrestrial invertebrate groups to their avian consumers could be identified using stable carbon or nitrogen isotope studies, particularly if there are differences in diet covering a wide range of trophic levels (i.e., detritivory, herbivory, predation) and corresponding differences in the isotope signature of food sources (i.e., plants, algae, aquatic or terrestrial detritus, or other arthropods).

Since filling of Kesterson, the largest and most persistent ephemeral pools have been filled with soil from interior berms or with spoils from KNWR (i.e., for the large pool located in the southeast corner of Pond 11). This has helped to reduce the extent and duration of ephemeral pools and breeding-season use by aquatic birds has been limited. However, late rains or a cool, wet spring could cause ephemeral pools to last into the breeding season. At the Se concentrations found in water and associated aquatic invertebrates, this could put aquatic birds utilizing these pools at risk. If this situation were to develop and monitoring determined significant use of ephemeral by aquatic birds, management activities such as hazing would be implemented (USBR, 1995).

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Section 4

Bird Nesting and Reproduction

Introduction

Reproductive effects of Se have been observed in wild aquatic birds at Kesterson in the past. Effects have included embryo mortality and teratogenesis, and failure of adult birds to breed (Ohlendorf et al., 1990; Skorupa and Ohlendorf, 1991). Monitoring of nests has been conducted since 1988 and has shown continued elevated Se concentrations in eggs but no evidence of Se-caused reproductive effects.

Nest boxes were set up at Kesterson in 1995 to study starlings, kestrels, and barn owls. Egg Se concentrations and success of the nests in boxes are included in this section for comparison to other birds nesting at Kesterson. However, the results of the nest box studies are discussed in greater detail in Section 5.

Objectives

The objectives of this study were to determine Se concentrations in bird eggs and to assess the reproductive success of birds at Kesterson Reservoir. Reproductive success was evaluated by determining the frequencies of embryonic mortality and developmental abnormalities, as well as hatching and fledging success of birds nesting during spring and summer of 1997.

Methods

Nest Searches

From mid-February through June 1997, biologists searched for and monitored, on a weekly basis, nests of shorebirds, waterfowl, and terrestrial species at Kesterson. Most shorebird (killdeer) nests were located by driving slowly along the levee roads and watching for the

adult leaving the nest, displaying, or "sneaking" away from the nest. Other bird nests were located by looking for birds displaying behaviors associated with nesting activities and opportunistically finding nests while conducting surveys and other activities.

Nest Monitoring

After a nest was found, it was marked with flagging approximately 10 feet away from the nest, mapped, and given a unique code. Nest codes include four-letter species acronyms, the pond number, and a nest number. Monitoring of the nest began immediately after its discovery and continued on a weekly basis to assess hatching success and nestling survival. Monitoring was conducted with minimum disturbance to the nesting birds. Each time the nest was visited the date, nest code, and number of eggs were recorded on data sheets. A nest was considered unsuccessful if all eggs failed to hatch (Mayfield, 1975; Ohlendorf et al., 1989). Nests were considered abandoned if a clutch was not completed and nests appeared unattended on subsequent visits and eggs were cold. Predation was determined by disappearance of eggs from a nest before the expected hatching date or observation of partially eaten eggs or egg contents in the nest.

Egg Collections

One egg was removed immediately after a nest was located if the nest contained more than one egg. At collection, each egg was marked with its unique nest code and the date it was removed. The data were recorded on a data sheet and the egg was placed in a container to avoid damage. All eggs removed from nests were either examined or refrigerated within 1 hour of collection.

Laboratory Examinations

Each egg was opened as soon as possible after collection to determine its fertility, stage of development, the position of the embryo, and whether pipping had occurred. Each embryo was examined for evidence of external deformities. The entire egg contents were then saved in chemically cleaned containers and frozen until shipment to the laboratory for Se analysis.

Results

Table 4-1 presents a summary of data from all nests observed at Kesterson in 1997 and the Se concentrations in eggs analyzed.

Table 4-1. Fate and Se Concentration (ppm, dry wt.) in Eggs of Nests Monitored at Kesterson Reservoir, 1997

Species	Location[a]	Date Found	Egg Collected	Eggs in Clutch	Viable	Whole Egg Se	Nest Fate
American kestrel	Pond 1	4/26/97	Yes	5	V	3.1	Successful, all remaining eggs hatched
American kestrel	Pond 8	5/17/97	Yes	5	V	3.7	Successful, all remaining eggs hatched
American kestrel	Pond 1	6/7/97	Yes	5	V	4.0	Successful, all remaining eggs hatched
Barn swallow	Pond 7	3/23/97	Yes	5	A	8.5	Successful, all remaining eggs hatched
Barn swallow	Pond 4	7/10/97	Yes	3	B	4.2	Unknown
Barn swallow	Pond 2	7/10/97	No	--	V	--	Successful
European starling	Pond 1a	4/8/97	Yes	3	V	2.6	All eggs collected
European starling	Pond 1b	5/8/97	Yes	5	V	3.2	Predation
European starling	Pond 2a	4/24/97	Yes	6	V	7.2	Successful, all remaining eggs hatched
European starling	Pond 2b	5/28/97	Yes	5	V	3.6	All eggs collected
European starling	Pond 2	5/28/97	Yes	5	V	3.4	All eggs collected
European starling	Pond 4a	4/24/97	Yes	4	V	4.5	All eggs collected
European starling	Pond 4b	5/28/97	Yes	4	V	3.4	All eggs collected
European starling	Pond 4b	5/28/97	Yes	4	V	4.1	All eggs collected
European starling	Pond 5	4/24/97	Yes	4	V	5.0	All eggs collected
European starling	Pond 6a	4/8/97	Yes	6	V	2.8	All eggs collected
European starling	Pond 6b	5/8/97	Yes	5	V	4.5	All eggs collected
European starling	Pond 7a	5/7/97	Yes	5	V	2.9	All eggs collected
European starling	Pond 7b	5/7/97	Yes	5	V	5.2	All eggs collected
European starling	Pond 7c	5/28/97	Yes	5	V	4.9	All eggs collected
European starling	Pond 8	4/24/97	Yes	3	V	3.6	All eggs collected
European starling	Pond 9	5/28/97	Yes	4	V	3.0	All eggs collected
European starling	Pond 10	4/24/97	Yes	4	V	2.9	All eggs collected
European starling	Pond 11a	4/29/97	Yes	4	V	2.7	All eggs collected
European starling	Pond 11a	4/29/97	Yes	4	V	3.5	All eggs collected
European starling	Pond 12a	4/24/97	Yes	5	V	3.3	All eggs collected
European starling	Pond 12b	4/24/97	Yes	6	V	2.4	All eggs collected
European starling	Pond 12c	5/28/97	Yes	6	V	2.0	All eggs collected
European starling	Pond 12c	5/28/97	Yes	6	V	2.2	All eggs collected

Table 4-1. Fate and Se Concentration (ppm, dry wt.) in Eggs of Nests Monitored at Kesterson Reservoir, 1997

Species	Location*	Date Found	Egg Collected	Eggs in Clutch	Viable	Whole Egg Se	Nest Fate
European starling	Off-site	4/97	Yes	--	V	2.5	All eggs collected
European starling	Off-site	5/97	Yes	--	V	4.9	All eggs collected
Ash-throated flycatcher	pond 6	7/97	No	3	V	--	Successful, all eggs hatched
Killdeer	P 3/4/2	3/15	Yes	4	V	17	Predation
Killdeer	Pond 1	3/22/97	Yes	4	V	2.4	Predation
Killdeer	Pond 3	3/23/97	Yes	4	V	64	Predation
Killdeer	Pond 12	3/24/97	Yes	4	V	2.3	Successful
Killdeer	Pond 1	3/15/97	Yes	2	V	20	Predation
Killdeer	Pond 9	2/26/97	Yes	4	F	13	Predation
Killdeer	Pond 3	3/15/97	Yes	4	V	58	Predation
Killdeer	Pond 1	4/3/97	Yes	4	V	3.5	Successful
Killdeer	Pond 1	5/1/97	Yes	4	V	3.9	Successful
Killdeer	Comp	4/4/97	Yes	4	V	17	Successful
Killdeer	SLD	4/22/97	Yes	4	V	3.3	Predation
Killdeer	Pond 1	4/2/97	Yes	4	V	4.9	Successful
Killdeer	Pond 3	4/26/97	Yes	3	V	36	Predation
Killdeer	Pond 1	4/17/07	Yes	4	V	3.8	Successful
Killdeer	Pond 11	4/9/97	Yes	4	V	4.5	Successful
Killdeer	Pond 11	4/22/97	Yes	4	V	8.7	Unknown
Killdeer	Pond 12	4/9/97	Yes	4	V	1.8	Successful
Killdeer	SLD	2/26/97	Yes	4	V	4 1	Predation
Killdeer	Pond 10	4/3/97	Yes	2	V	4.7	Predation
Killdeer	SLD	4/27/97	Yes	3	V	4.1	Successful
Killdeer	Pond 10	5/15/97	Yes	2	V	3.3	Abandoned
Killdeer	Pond 12	5/17/07	Yes	4	V	3.3	Successful
Killdeer	Pond 12	5/20/97	Yes	2	V	3.6	Destroyed by road grader
Killdeer	Pond 7	5/28/97	Yes	4	V	9.1	Successful
Killdeer	Pond 1	5/29/97	Yes	4	V	2.8	Destroyed by road grader
Killdeer	Pond 1	5/30/97	Yes	4	V	2.6	Predation
Killdeer	Pond 1	5/30/97	Yes	4	V	3.2	Predation
Killdeer	Pond 11	5/30/97	Yes	4	V	2.7	Predation
Killdeer	SLD	6/11/97	Yes	4	V	3.2	Destroyed by road grader
Killdeer	Pond 12	6/27/97	Yes	3	V	3.4	Predation
Killdeer	Pond 2	6/29/97	Yes	4	V	5.4	Unknown
Killdeer	Pond 3	6/30/97	Yes	4	V	3.2	Destroyed by road grader

Table 4-1. Fate and Se Concentration (ppm, dry wt.) in Eggs of Nests Monitored at Kesterson Reservoir, 1997

Species	Location[a]	Date Found	Egg Collected	Eggs in Clutch	Viable	Whole Egg Se	Nest Fate
Killdeer	Pond 1	6/30/97	Yes	4	V	3.1	Unknown
Killdeer	Pond 1	6/30/97	Yes	3	V	3.6	Unknown
Killdeer	Pond 5	6/27/97	Yes	3	V	8.3	Unknown
Mallard	Pond 3	4/4/97	Yes	6	V	1.6	Predation
Mallard	Pond 1	5/16/97	Yes	6	V	2.4	Predation
Mallard	Pond 1	5/21/97	Yes	8	V	3.4	Predation
Mallard	Pond 6	7/10/97	No	7	--	--	Predation
Mourning dove	Pond 1	3/23/97	Yes	2	V	1.6	Predation
Mourning dove	Pond 1	4/2/97	Yes	2	B	1.3	Predation
Mourning dove	Pond 4	4/24/97	Yes	2	V	2.0	Abandoned
Barn owl	Pond 4	3/28/97	Yes	5	V	8.0	Successful
Red-tailed hawk	Pond 11	3/97	No	0	--	--	Abandoned prior to completion
Red-tailed hawk	Pond 2	3/97	No	0	--	--	Abandoned prior to completion
Nighthawk	Cmpnd	6/97	No	2	V	--	Successful
Nighthawk	Pond 1	6/27/97	Yes	2	V	4.3	Predation

[a] Eggs from locations sharing the same letter are from the same clutch, those from the same location with a different letter refer to additional clutches in the same nest box

V- viable; A- egg addled; B-egg broken, F- fertile egg

Embryo Ages

Eggs were collected as soon as a nest was discovered to avoid loosing samples to predation and to maximize the number of nests sampled for Se. Embryos were considered viable if the egg was fertile or a living embryo could be observed. Most eggs contained embryos under 10 days old, before most abnormalities characteristic of Se-toxicosis can be observed. Embryo mortality is not distributed randomly during incubation. of the egg. There are generally two embryo mortality peaks, one at about 2 to 3 days of development and a second, more pronounced mortality peak between 18 and 20 days of development. These peaks coincide with fundamental changes in physiological functioning of various parts and organs (Landauer, 1967). Because most eggs were collected early during incubation, viability is likely over-represented (i.e., many eggs had early embryos that might have died in the later "mortality peak") for samples collected in 1997.

Barn Swallow

Two eggs were collected in 1997 (Table 4-1). Nests are usually found in the culverts along the south side of Gun Club Road. However, few nests have been found in these culverts since 1994. Only one nest was found in 1995, and two nests were found in 1996 and again in 1997. A third nest was found under the metal San Luis Drain bridge adjacent to Pond 7. The eggs collected in 1997 had Se concentrations similar to concentrations found in swallow eggs collected from Kesterson in other years (Table 4-2).

Table 4-2. Se Concentrations (ppm, dry wt.) in Barn Swallow Eggs Collected From Kesterson Reservoir

Year	*n*	Range	Geometric Mean[a]	Comments
1988	33	3.8 - 11	6.6	Prior to dewatering of Kesterson
1989	13	4.0 - 7.9	5.6	Kesterson dewatered in fall of 1988, many nest sites (culverts) removed.
1990	9	3.7 - 6.3	5.6	
1991	9	4.1 -6.8	5.2	
1992	3	4.2 - 6.1	4.8	
1993	4	5.1 - 5.6	5.3	Heavy March rain destroyed some nests
1994	5	5.4 - 11	7.5	
1995	1	5.3	-	
1996	1	7.4	-	
1997	2	4.2 - 8.5	6.0	

[a]There was no significant difference in Se concentrations among years.

European Starling

Twenty-three clutches were laid in nest boxes on Kesterson by starlings and an egg was analyzed for Se from each clutch (Table 4-1). Starlings are probably a good feeding-guild surrogate for western meadowlarks (a common native species; see Section 6). All of the eggs collected contained viable and normal embryos. Ten of the nests were located along the San Luis Drain and the remainder were located in interior locations of Kesterson (see nest box location map in Section 5, Figure 5-1). Se concentrations in eggs were not significantly

different among years (Table 4-3) or among locations. We attempted to collect all eggs prior to hatching to increase nest box use by American kestrels and to limit reproduction by starlings (a non-native species).

Table 4-3. Se Concentrations (ppm, dry wt.) in European Starling Eggs Collected From Kesterson Reservoir

Year	*n*	Range	Geometric Mean*	Comments
1991	1	2.8	2.8	
1996	30	1.5 - 13	3.7	First year that nest boxes were available
1997	23	2.0 - 7.2	3.4	Eggs removed prior to hatching

*There was no significant difference in Se concentrations among years.

Killdeer

Thirty-five killdeer nests were found and an egg was collected from each nest (Table 4-1). Twenty-one of these nests were found along the road on the west side of the San Luis Drain (the east side of Kesterson), nine were found on interior roads within Kesterson, one was found on the edge of Reclamation's compound between Pond 4 and the Fremont Canal, and four were found on the drain road south of Kesterson between the reservoir and Highway 165. Killdeer nests were found from February through June, 1997: Two nests were found in February, six nests were found in March, eleven nests were found in April, nine nests were found in May, and seven were found in June. Eleven of the nests were found empty at the time the eggs were expected to hatch with no signs of predation and were assumed to have been successful. Fourteen nests were unsuccessful due to predation, four nests were destroyed when the road was graded, and one nest was abandoned. There were no eggs or evidence of predation found at five killdeer nests that were not monitored during the week before they were expected to hatch and there fate is unknown. Se concentrations in the killdeer eggs collected from Kesterson ranged from 1.8 to 64 ppm, with concentrations exceeding 10 ppm in seven eggs, all of which were collected in February, March, or April (during this time ephemeral pools were present and killdeer may have been feeding on greater numbers of aquatic invertebrates). The geometric mean concentration of Se in 31 killdeer eggs collected from in or adjacent to Kesterson during 1997 (5.8 ppm) was higher

than the level found in the four eggs collected along the San Luis Drain south of Kesterson (3.7 ppm). The mean concentration of all killdeer eggs collected from in or adjacent to Kesterson during 1997 was similar to concentrations in killdeer eggs collected from 1989 through 1994, and significantly below that of killdeer eggs collected in 1988 (Table 4-4).

Table 4-4. Se Concentrations (ppm, dry wt.) in Killdeer Eggs Collected From Kesterson Reservoir

Year	*n*	Range	Geometric Mean*	Comments
1988	24	6.5 - 58	15.8 A	Prior to dewatering of Kesterson
1989	9	3.5 - 15	6.4 B	Kesterson dewatered in fall of 1988
1990	11	2.9 - 20	7.0 B	
1991	7	3.1 - 12	5.4 B	
1992	13	2.5 - 28	8.5 AB	
1993	2	11 - 17	13.7 AB	Heavy March rain interrupted nesting
1994	12	2.8 - 11	5.6 B	
1995	12	2.3 - 13	5.7 B	
1996	20	2.2 - 13	5.4 B	
1997	29	1.8 - 64	5.6 B	Interior and west-side nests found

*Geometric means sharing the same letters are not significantly different (Tukey HSD $P \leq 0.05$).

Mallard

Four mallard nests were found in 1997: one in April, two in May, and one in July. Eggs were analyzed from three nests (Table 4-1). All the mallard nests found in 1997 were lost to predation. Fewer waterfowl nests have been found at Kesterson since it was dewatered in the fall of 1988 and few of those nests that have been found were successful despite relatively low Se concentrations in most analyzed eggs. There were no significant differences in Se concentrations found in mallard eggs collected from 1988 to 1997 (Table 4-5).

Table 4-5. Se Concentrations (ppm, dry wt.) in Mallard Eggs Collected From Kesterson Reservoir

Year	*n*	Range	Geometric Mean*	Comments
1988	15	3.9 - 31	12	Prior to dewatering of Kesterson
1989	1	3.7	-	Kesterson dewatered in fall of 1988
1990	2	1.7 - 3.1	2.3	
1992	1	1.1		
1993	4	1.9 - 15	5.8	Heavy March rain
1994	1	7.7	-	
1995	3	4.5 - 16	8.9	
1996	3	1.6 - 5.3	2.8	
1997	3	1.6 - 3.4	2.4	

*There was no significant difference in Se concentrations among years.

Mourning Dove

Three mourning dove nests were found in 1997 (Table 4-1). A single egg was collected from a nest located in the Compound that was later abandoned and two eggs were collected from nests located in Pond 1 that were later lost to predators. Mourning dove eggs collected in 1997 had Se concentrations similar to (uniformly low) concentrations found in other eggs from Kesterson collected in 1988 and 1989 (Table 4-6).

Table 4-6. Se Concentrations (ppm, dry wt.) in Mourning Dove Eggs Collected From Kesterson Reservoir

Year	*n*	Range	Geometric Mean*	Comments
1988	2	1.6 - 1.7	1.6	Prior to dewatering of Kesterson
1989	2	1.3 - 4.7	2.5	Kesterson dewatered in fall of 1988
1997	3	1.3 - 2.0	1.6	

*There was no significant difference in Se concentrations among years.

Lesser Nighthawk

Two lesser nighthawk nests were found and monitored in 1997 (Table 4-1). One nest was found on the gravel roadway along the San Luis Drain adjacent to Pond 1 and the other on a

gravel area in the USBR trailer compound (Compound). The Compound nest had two chicks in it when it was discovered and one egg was collected from the nest in Pond 1. The Pond 1 nest was lost to predators and the Compound nest successfully fledged two chicks. Se concentration in the egg analyzed was similar to the Se concentration found in the nighthawk egg collected from Kesterson in 1992.

Other Nests

Other nests that were found at the Reservoir in 1997 were red-tailed hawk, American kestrel (nest box), barn owl (nest box), ash-throated flycatcher (nest box), and tree swallow. Kestrel and barn owl nesting is discussed in Section 5. A red-tailed hawk nest was found on a power pole located on the San Luis Drain road in Pond 11 in the vicinity where red-tailed hawks have nested and attempted to nest in previous years. This nest was not completed for unknown reasons and no eggs were laid. A second red-tailed hawk nest was located on a power pole at the east end of Gun Club Road where a pair had attempted to nest in 1996. This nest was not very stable and it was again blown over by high winds before any eggs had been laid.

Discussion

Geometric mean Se concentrations were similar among species collected from Kesterson in 1997 (Table 4-7). Because much of Kesterson may be marginal nesting habitat for many birds, most may nest on the periphery of Kesterson and may feed off-site much of the time. Off-site feeding may partly account for lower Se concentrations in eggs collected from the San Luis Drain levee than those collected from the interior of Kesterson.

Table 4-7. Summary of Se Concentrations (ppm dry weight) In Bird Eggs Collected From Kesterson Compared by Species, 1997

Species	*n*	Geometric Mean[b]	Range
Barn swallow	2	7.4	4.2 - 8.5
European Starling	25	3.4	2.0 - 7.2
Lesser nighthawk	1	4.3	4.3
Mourning dove	3	1.6	1.3 - 2.0
Mallard	3	2.4	1.6 - 3.4
Killdeer[a]	29	5.2	1.8 - 64
American kestrel	3	3.6	3.1- 4.0
Barn owl	1	8.0	8.0

[a] Only eggs from nests found on Kesterson are included in this table

[b]There was no significant difference in Se concentrations among species.

Since 1993, when heavy rain early in the nesting season for killdeer interrupted nesting, the number of killdeer nests found at Kesterson has increased and nests were found in higher numbers in 1997 than in other years. Nest initiation was also earlier in 1997 compared with 1995 and 1996 and nesting ended later than in 1995 (Figure 4-1). However, fifty percent of the nests had been started by the end of April in all three years. In 1997, killdeer nests started in February through April had significantly higher mean Se concentrations than eggs collected in May through June. In previous years, there were no similar significant differences in Se concentrations in killdeer eggs collected during these time periods. A high percentage of nests (30 percent) in 1997 were located on interior roads and along the roads on the west side of Kesterson (Interior eggs). These eggs had a higher geometric mean Se concentration than those from along the east side of Kesterson. In previous years the number of nests found were roughly proportional to the number of killdeer using the reservoir (about 2 nests for each killdeer), based on daily use surveys during the breeding period (February through June). This is illustrated in Figure 4-2. However, in 1996 and 1997 the ratio was about three to one. One reason why more nests are found on Kesterson than can be accounted for by killdeer population on the reservoir may be that some of the killdeer

foraging in the Kesterson National Wildlife Refuge (KNWR) and other areas adjacent to Kesterson use the reservoir for nesting. It is also possible that behavior of birds while foraging in the ponds makes them less visible than their nesting on the roads (preferred nesting sites). Another possible reason for the increase in killdeer nests is that the vegetation structure is changing due to edaphic factors or succession and there are more areas with short vegetation suitable for killdeer nesting within Kesterson.

Figure 4-1. Cumulative Probability of Clutch Initiation by Killdeer

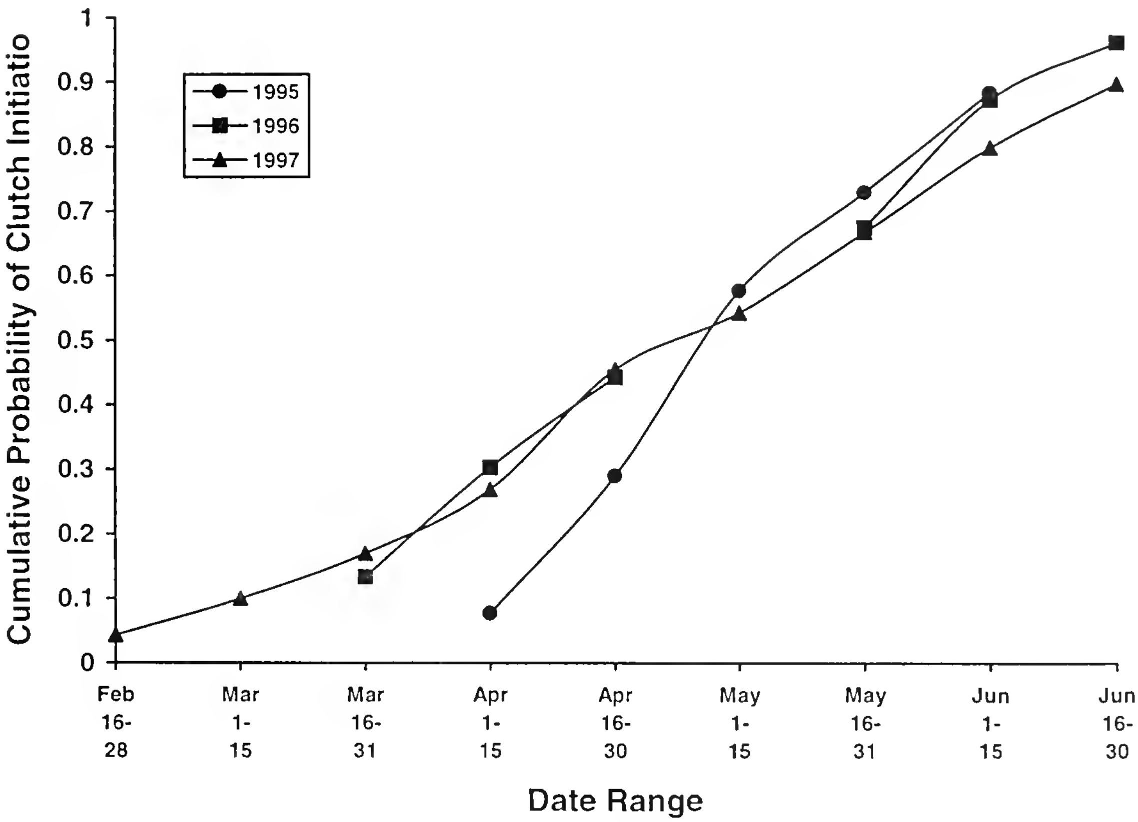
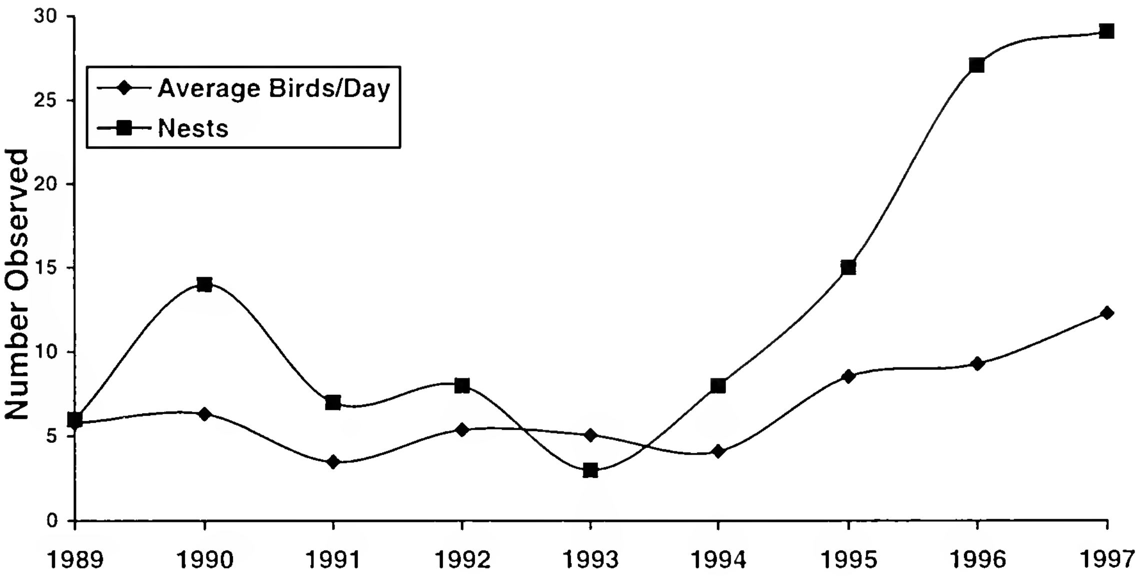


Figure 4-2. Killdeer Average Daily use During the Breeding Season (February through June) and Number of Nests Found



Other than killdeer and starlings, there were relatively low numbers of nests found at Kesterson in 1997. As in other years, there was a high percentage of nest predation among ground-nesting species. Poor nesting success for ducks and shorebirds may be due to physical factors such as the loss of habitat as ephemeral pools dry(this has been observed in other years). Prior to dewatering of Kesterson in 1988, many upland areas were at least partially protected from predators by being surrounded by water during the reproductive period and the types of plants were, for the most part, low growing species. Since Kesterson was dewatered, conditions for nesting for the various bird species have changed and, in general, Se concentrations in eggs have gone down (see Tables 4-2 through 4-6). Conditions for nesting have changed due to a number of factors such as variations in climatic conditions (e.g., rainfall and temperature), conversion of Kesterson from a wetland to a terrestrial ecosystem, successional changes in vegetation causing plant species changes and structural changes in habitat, and disturbance during the reproductive season from monitoring and management activities.

Nest sites on Kesterson are limited for some species. Birds such as mallards and black-necked stilts now have less wetland habitat to attract them. Barn swallows that use concrete

culverts for nesting are limited by the number of these structures left at Kesterson (many of these culverts were removed during filling of Kesterson). Also, many barn swallow nests have fallen down during heavy rainfalls and have not been rebuilt. Killdeer prefer open areas around their nests, and the tall weeds and grasses that currently grow at Kesterson make much of the interior of the site unsuitable for killdeer or other shorebirds nesting. However, conditions in 1997 were more suitable for nesting in the interior possibly due to the late rains which kept the vegetation low until late in the summer.

Of the killdeer eggs collected at Kesterson, the percent of nests lost to predation (40-percent) was slightly higher than the percent of successful nests (33-percent). This was similar to the predation rate of 36-percent from 1984 - 1985 at Kesterson (Ohlendorf et al., 1989). Se concentrations in eggs collected from killdeer nests in the interior and west side were significantly higher than concentrations in eggs collected from the east side (i.e., along the San Luis Drain). Eggs collected from the interior and west side of Kesterson had a geometric mean of 12 ppm (N = 10; Range = 2.4 - 64 ppm) and these eggs had a higher geometric mean Se concentration ($P = 0.0425$) than those collected along the San Luis Drain on the east side of Kesterson (GM = 4.2; N = 21; Range = 1.8 - 20 ppm). Although the geometric mean Se concentration in killdeer eggs (5.8 ppm) was below the suggested level of concern for clutch viability for stilts (6 - 15 ppm; Skorupa et al., 1996), individual eggs were above the concentrations expected to produce embryo toxicity and teratogenesis. Se-related effects in bird reproduction at Kesterson have not been found since the filling operation. Many eggs continue to have Se levels above median background levels of 1.9 ppm and 21 of the 79 eggs collected had Se concentrations above the 4.5 to 5.0 ppm threshold level for above background contamination for the region (Joseph Skorupa, USFWS, Personal Communication). Seven killdeer eggs were above the suggested avian threshold for reproductive impairment of 10 ppm (Heinz, 1996). Six killdeer eggs were above the 14 ppm EC_{01} (Effect Concentration for 1-percent of stilt eggs). Although killdeer are probably more sensitive than tolerant taxa (i.e., American avocet), two killdeer eggs collected in 1997 were above the threshold level for embryo teratogenesis for tolerant taxa (50 ppm; Skorupa et al., 1996). The highest Se levels found in killdeer eggs collected in 1997 (58 and 64 ppm) are

high enough to expect that embryo teratogenesis could occur in this species at Kesterson. Thus, the potential for Se-induced reproductive problems continues to exist there.

There are few nest sites for red-tailed hawks and great horned owls at Kesterson and the available sites (e.g., power poles) do not afford the same protection from severe weather conditions as do natural nest sites (e.g., trees). No northern harrier or great horned owl nests were found in 1997. Barn owls and kestrels used artificial nest boxes.

Although there were at least three pairs of red-tailed hawks that remained at Kesterson through the breeding season in 1997, only two early nesting attempts were made. It is not known why these pairs did not nest this year and it is unusual for paired red-tails to remain in an area without nesting. However, red-tailed hawks successfully nested and fledged chicks from a tree-nest in KNWR adjacent to Kesterson in 1997. It is possible that the nesting substrate at Kesterson (i.e., power poles) is not adequate for nesting, although red-tailed hawks have used them with some success in the past.

To be sure that sampling represented Se concentrations for Kesterson, a random egg was collected from each nest found. Eggs were collected early during the incubation period from most nests so that as many nests as possible would be sampled (i.e., few nests were not sampled due to predation of the nest prior to collecting an egg). This provides an accurate representation of Se concentrations for the entire site and helps to identify possible "hot spots" for management or additional sampling. However, collecting early during the incubation period increases the number of viable eggs (i.e., fertile eggs or those with living embryos) because there is potentially higher mortality later during incubation and embryo teratogenesis is more likely to be found in later-stage embryos.

To address this problem of collecting eggs early enough to avoid losses from predation and also collecting samples that are likely to show embryo mortality or teratogenesis (if it is occurring), two eggs should be collected from each shorebird nest. However, predation is typically high in killdeer nests (Table 4-1). Many of the high-Se killdeer nests in 1997 were found in the interior of the site where predation may be high. This may bias the results if a disproportionate number of high-Se nests experience predation. Monitoring solutions will be discussed with the Service to minimize any bias.

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Section 5
Nest Box and Telemetry Study

Introduction

To date, adverse reproductive effects in terrestrial birds have not been observed in the course of avian monitoring at Kesterson. Based on monitoring data alone it is not possible to distinguish whether this lack of effect is due to: 1) Se ingestion being relatively low because nesting birds forage primarily offsite; 2) selective foraging on dietary items low in Se; and/or 3) reduced sensitivity to Se compared to aquatic species such as ducks and stilts. In order to address some of these important data gaps, artificial nest boxes were placed on and around Kesterson during the late winter-early spring period in 1996 to attract cavity-nesting birds. Under these circumstances individual birds can be studied closely to provide site-specific information on exposure to, and the effects of, high dietary Se concentrations. For the purpose of comparison with the kestrel laboratory feeding study, and because it is known to utilize Kesterson, the target species in this study is the American kestrel (*Falco sparverius*). However, other cavity-nesting bird species found at Kesterson can also provide useful data. In 1996, only European starlings (*Sturnus vulgaris*) occupied the nest-boxes and they were sampled extensively for selenium in tissues and for dietary composition analysis (see CH2M HILL 1996). Nest-box studies have been used by others (e.g., Grue and Christian, 1981) to study effects of contaminants on avian reproductive success.

In the case of nest boxes that become occupied by kestrels, foraging ranges of breeding birds can be estimated using radiotelemetry to monitor bird movements. This potentially allows determination of the extent to which the birds forage at Kesterson and estimation of dietary Se exposure, both of which can be compared with the reproductive success of the birds. In addition, analyses of regurgitated pellets from American kestrels can provide an approximate prey composition for this species and improve dietary Se estimates. Blood sampling of

breeding birds and their offspring for Se analyses, together with bioaccumulation data from the laboratory kestrel feeding study, will be used to further characterize exposure patterns.

In addition to the kestrel/starling nest boxes, a single barn owl (*Tyto alba*) nest box was placed in the fenced compound adjacent to the west edge of Pond 4 (Trisection 1) in 1996. Five barn owl chicks fledged successfully from this box in 1996. Barn owls are common all year at Kesterson, and have nested there previously. In California, this species will breed year-round when conditions permit (Marti 1992).

Methods

Setting Up the Nest Boxes

On February 7 through 9, 1996, 30 kestrel nest boxes were set up on the reservoir. They were placed in lines running east-west, approximately 1/4 mile apart. Eleven of the boxes were fastened to existing power poles within Kesterson (eight nest boxes were located along the San Luis Drain and three along Gun Club Road) and 19 were fastened to 12 ft pressure-treated 4x4s sunk about 3 ft into the ground. The posts were tamped in and not cemented. At each of these locations the posts were placed on the highest point in the area to gain additional elevation. Wood shavings were added to each box to line the bottom with at least three inches of shavings. Each boxes was given a unique code. Codes identified type of nest (NB), where the box was located (Pond 1 through 12 or State Route 140 or 33) and a series beginning with the number 1 for each pond. For example NB01-1 identifies a nest box in the northeast corner of Pond 1 and NB01-4 identifies a nest box in the southwest corner of Pond 1. Aluminum tags with the number were attached to each nest pole and the location and number were identified on a map.

On March 1, one more box was fastened to an existing pole in the northwest corner of Pond 4 (Trisection 1). Five boxes were also installed outside the reservoir: two along Highway 140 by the main entrance to Kesterson National Wildlife Refuge (KNWR), and three on Highway 165 from the east gate of KNWR (i.e., the intersection of Highway 165 and the San Luis

Drain) south. Four of these were mounted on existing poles and one was attached to a eucalyptus tree.

The nest boxes were made of redwood, about 1 1/2 ft tall, 1 ft deep, and 1ft wide. The lids were either hinged or totally removable with hook and eye clasps to lock them on. Boxes were ventilated through the entrance hole and through holes drilled in the bottom.

Nest Box Monitoring

Pairs of kestrels were observed for signs of nesting beginning in early March of 1997. Established pairs were identified by observations of kestrels entering and leaving nest-boxes, nest territory defense behavior, and food deliveries by the male to the female at the box. Nest boxes were checked at several times during the reproductive period: during egg-laying or early incubation when one egg was collected for viability assessment and Se analysis; during late chick rearing, when chicks were assessed for overall health/normality, banded (USFWS bands) and blood sampled; and during post-fledging, when fledging success was determined (absence of chicks and/or chick remains in or near the box was assumed to indicate successful fledging). Capture of the adult female in the nest box was attempted during early incubation, after egg-laying was complete; females were blood sampled, banded (USFWS bands), and placed back in the nest box within approximately 30 minutes of capture. Nest boxes that were apparently occupied by kestrels, but then abandoned, were also checked to assess stage of reproduction at which abandonment occurred and fate of the adult kestrels.

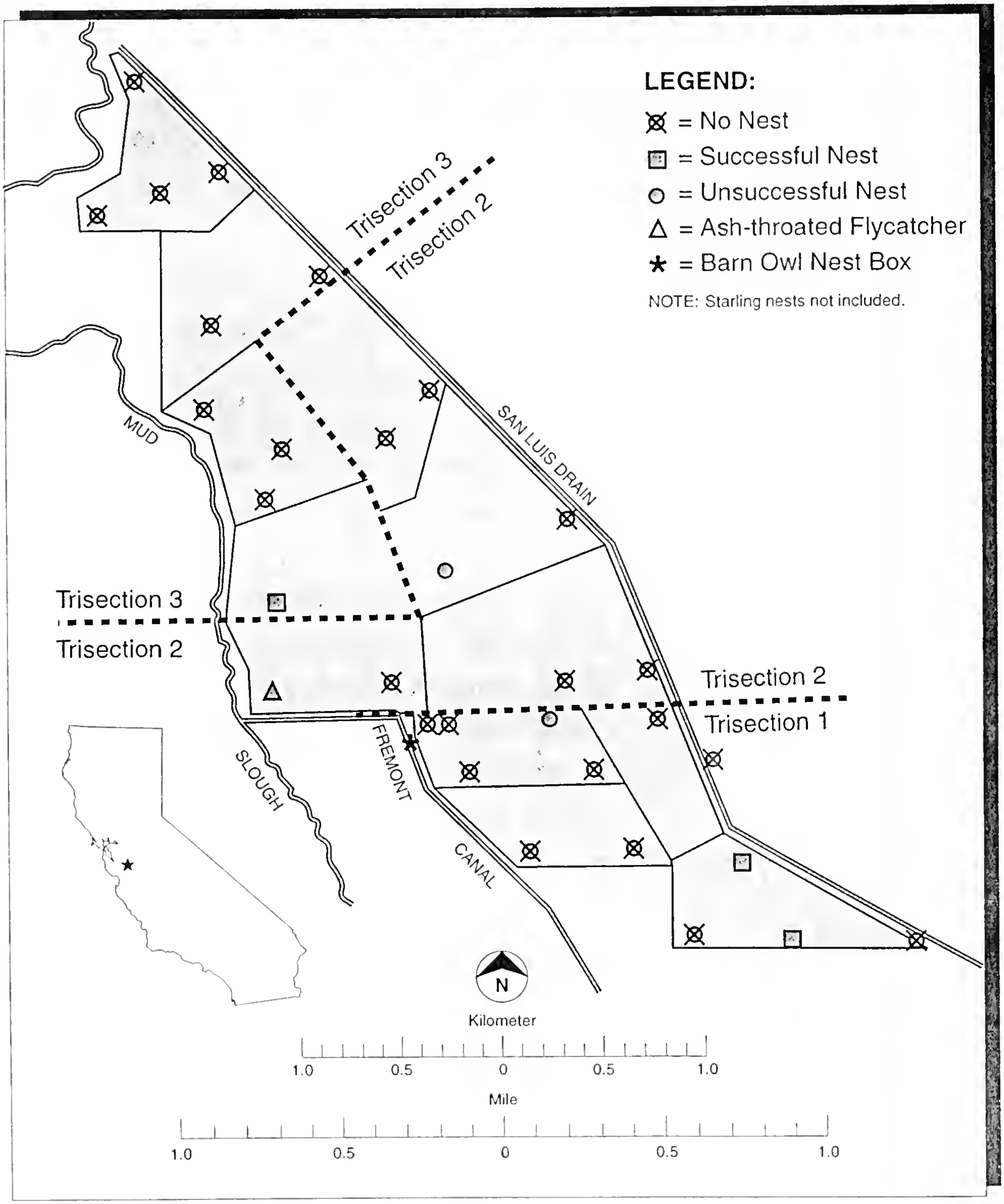

Figure 5-1
Approximate Locations and Fate of Nest Boxes on Kesterson Reservoir

CH2MHILL

Radiotelemetry Data Collection

After establishment of the nest and during the incubation phase of reproduction, attempts were made to trap the males of these pairs using dho-gaza or bal-chatri trapping methods. Trapped birds were banded with a USFWS band, and blood sampled. Prior to release, transmitters weighing approximately 2 g with 5-month battery life (Holohill, Inc., Canada) were attached as rump packs with 1/4 inch teflon webbing sewn with cotton thread. Radiotagged birds were released at the capture site.

Radiotagged birds were tracked by car and foot approximately three days per week, using a hand-held yagi antenna, and an ICOM (Model IC H-16) receiver. Attempts to locate individual birds were made at approximately 2-hour intervals throughout each tracking day between the hours of 0600 and 1800. Locations were visual (with binoculars) if possible, but were estimated using triangulation when necessary. Observers in cars maintained a distance of about 1/4 mile from birds to minimize the influence of observers on bird movement. Each radiotagged bird was tracked from the time of capture until the signal ceased (transmitter failure or dispersal away from the area by the bird) or fledging of kestrel chicks, whichever occurred sooner.

Each time a bird was located, its position was noted on a map and denoted with an identifying number, which corresponded to data for that observation on a separate data sheet. In addition to location, other variables were recorded: time, whether observation was visual or auditory (triangulation), whether the bird was perched or flying, perch type (wire, pole, tree, etc.), activity (hunting, social interactions), and weather conditions at the time of the observation. All activities were recorded for five minutes after locating a bird. Changes in position during this period were recorded as "activities" and not as location data. Location data were transformed into latitude-longitude in the field using a hand-held global positioning unit.

Pellet Analysis

Kestrel pellets from Kesterson were collected opportunistically during the breeding period in and under nest boxes and nearby perches utilized by the adult birds. Kestrel pellets are distinguishable from those of other raptors at Kesterson by their small size. Barn owl pellets and other food remains were collected from the single barn owl nest box, after the young had fledged. The top (i.e., most recent) layer was separated to attempt to determine if food items caught after the chicks hatched were the same as those prior to hatching. For dietary component analysis, collected pellets were soaked in water, and bones, teeth, chitin and other non-digested parts were separated from fur and feathers. Food items were identified by distinctive features of the skull, mandible, teeth, head (insects) and other parts. Since barn owls typically swallow their prey whole, the approximate number of animals consumed could be estimated (Yalden and Morris, 1990; Marti, 1987, 1992). The number of animals was estimated by counting paired parts and each unique part as a single animal. Components of kestrel pellets were analyzed in terms of relative contribution (by converting the frequency of occurrence to percent relative biomass) of dietary components to the total pellet mass.

Results

Reproductive Success

Five nest boxes on Kesterson (16% occupancy) and three reference boxes (60% occupancy) were used by American kestrels during the breeding season. Kesterson nests were located in interior locations in Ponds 4, 7, and 8, and on the edge of the reservoir in Pond 1 (two nests). Two off-site nests were located north of Kesterson near the entrance to the KNWR and another was located at the south end of the refuge.

Three of the five nests on Kesterson (Ponds 1 and 8) and all three of the reference nests successfully fledged young (Table 5-1). No developmental abnormalities were observed in kestrel chicks or in embryos of collected eggs from Kesterson or reference nests. Each of the two kestrel nesting failures on Kesterson was attributed to the death or disappearance of one

of the pair members prior to egg-laying. In neither case was loss of the adult bird believed to be selenium-related.

Table 5-1. Reproduction in kestrels using nest boxes on and off Kesterson Reservoir, 1997

Nest Location	Number of Nests	Mean Clutch Size	% of Incubated Eggs Hatched	% Developmental Abnormalities	% Successful Nests
Kesterson	5	5	100	0	60
Off-site	3	5	100	0	100

On March 28, 1997 five eggs were found in the barn owl box located in the storage compound west of Pond 4 (Trisection 1). One of these was collected for Se analysis, three hatched and one disappeared. On May 10, 1997 the three barn owl chicks were banded and the female parent was trapped in the nest box. Blood samples were taken from the adult barn owl and one of the chicks. The adult female had been previously banded and blood sampled in 1995.

Selenium Concentrations

Selenium concentrations in blood and eggs of kestrels that nested on Kesterson were variable and similar to those from off-site nests (Table 5-2). Adult (males and females pooled) and chick blood Se concentrations from both Kesterson and off-site nests were higher than those from reference areas in Yolo and Solano counties (CA) and captive control kestrels fed clean diets (data from 1994 and 1995 feeding studies; CH2M HILL, 1997a). Adult kestrels from Yolo and Solano counties also had higher blood Se concentrations than the captive control kestrels. Se concentrations were 3.8 ppm in the blood of the adult female barn owl, 8.0 in the egg, and 4.9 ppm in blood of the chick.

Table 5-2. Se Concentrations (ppm dry wt.) in American Kestrels and Eggs

	Egg Se			Adult Blood Se			Chick Blood Se		
Nest Location	GM	Range	*n*	GM	Range	*n*	GM	Range	*n*
Kesterson on-site	3.6 A	3.1 -4.0	3	4.8 A	3.0 -12	5	3.8 AB	3.2 -5.1	3
Off-site near Kesterson	2.4 B	1.8 -3.2	3	5.8 A	5.0 -6.8	3	5.3 B	2.9 -8.2	3
Yolo/Solano				3.3 B	2.0 -6.5	10	2.4 A	1.0 -4.5	7
Captive Control	2.1 B	1.8 -2.5	9	1.8 C	1.0 -4.0	253			

[a]Geometric means sharing the same letters are not significantly different (Tukey HSD $P \leq 0.05$).

Pellet Analysis

Composition of regurgitated pellets and calculated Se concentrations (based on 100% foraging at Kesterson) in kestrel and barn owl diets are shown in Table 5-3. Kestrel pellets from Kesterson consisted primarily of invertebrates, followed by small mammals. Barn owls fed primarily on small mammals; California voles made up the largest proportion of pellet remains. However, the species composition before and after hatching of barn owl chicks appeared to have changed from a diet dominated by California voles to one that included a larger proportion of deer mice and western harvest mice. This may reflect a change in preference or changed seasonal abundance. Calculated dietary Se (based on dietary composition and previously determined Se concentrations in dietary components) was higher for kestrels breeding on Kesterson than for the barn owls at the compound, mainly because of higher consumption by kestrels of insects which tend to have higher Se concentrations.

Table 5-3. Food Items Analyzed in Kestrel and Barn Owl Pellets and Se Concentrations

Location	Pellets Collected	Dietary Items Identified	% Composition (by volume)	GM Se Food Items[a] (ppm, dry wt.)	Estimated[b] GM Diet Se (ppm dry wt)
Kestrel	31	Insects	61	10	8.1
		Reptiles	5	5.9	
		Birds	11	6.1	
		Mammals	17	6.0	
		Non-animal	6		
Barn owl	Nest box contents	Bird	7	6.1	4.4
		CA vole	63	5.3	
		House mouse	1	8.3	
		Pocket gopher	20	1.8	
		Cottontail	4	3.6	

[a] Invertebrate samples collected from Kesterson in 1994 and 1995; reptile samples collected from Kesterson in 1990; bird samples from starlings collected at Kesterson in 1996; and small mammals collected from Kesterson in 1994, 1995, and 1996 (pocket gophers collected in 1989)

[b] Diet Se estimated using the proportion of food items from the pellet analysis.

Radiotelemetry Data

Male kestrels from three nest boxes (Ponds 1, 4, and 8) on Kesterson and one off-site nest box were successfully radio-tagged early in the breeding season (Table 5-4). Based on radiotelemetry observations, breeding male kestrels on Kesterson foraged about 58% of the time on-site. The male from the nest box positioned on the periphery of the reservoir (Pond 1 male; Bird 10) foraged less on-site than did the two males whose nests were located in the interior areas (Pond 4, Bird 2; Pond 8, Bird 7). The Pond 8 bird and the off-site bird were able to remove their transmitters after only several days of wear (this occurred due to a structural defect in the body of the transmitters). The Pond 4 bird did not remove his transmitter, but abandoned his territory after his mate disappeared (unknown cause) and left the Kesterson area. The Pond 1 bird was the only tagged bird for which it was possible to collect observations through the fledging period. Preliminary analyses of the spatial

distribution of observations indicate that maximum foraging distances for individual male kestrels ranged from 1.3 to 3.4 km from the nest box.

Table 5-4. Telemetry Observations of American Kestrels at Kesterson, 1997

Nest Location	Bird ID	# Days Observed	# Total Observations	% Observations on Kesterson	Maximum Foraging Distance (km)	Termination of Observations
Kesterson						
Pond 1	10	16	52	27	2.7	abandoned
Pond 4	2	27	80	71	3.4	removed transmitter
Pond 8	12	10	41	73	2.2	young fledged
Mean		*18*	*58*	*58*		
Reference	3	6	17	0	1.3	removed transmitter

Discussion

Among kestrel pairs that successfully laid eggs in this study, hatchability and fledging success were high both in Kesterson and off-site areas. Adult kestrel mortality was responsible for two early nesting failures on Kesterson. Because Se levels in kestrels at Kesterson (GM = 3.7 ppm [CH2M HILL, 1997b]) are not in range to cause adult mortality (10 - 15 ppm dietary exposure [Skorupa et al., 1996]), mortality was not believed to be directly attributable to Se exposure. Similarly, the single barn owl nest sampled resulted in 3 out of 4 remaining eggs (after one was collected) hatching and fledging successfully. The success of this nest was similar or higher than that of barn owls in southern California (P. Bloom, Western Foundation of Vertebrate Zoology, personal communication) and other barn owl populations (Marti, 1992). No developmental abnormalities were found among Kesterson and off-site nests, which is in agreement with previous nest monitoring data for other terrestrial species at Kesterson. These results are also consistent with the relatively low Se concentrations found in the collected eggs, all of which were below a suggested avian threshold for reproductive impairment of 10 ppm (Heinz, 1996).

Occupancy of nest boxes by kestrels was lower than expected. Potential limiting factors for increased occupancy include habitat quality, intraspecific food competition, interspecific nest box competition (commonly observed at Kesterson between kestrels and starlings), and disturbance (construction on the San Luis Drain may have caused the abandonment of a nest box in Pond 12 in 1996). Maximum foraging distances of kestrels, based on radiotelemetry observations, are consistent with previously reported home range information for kestrels (Balgooyen, 1976; Rudolph, 1982). Based on the results of other studies (Bechard and Bechard, 1996), it is expected that in 1998 there will be at least the same, and possibly a greater, number of kestrel pairs using nest boxes on and off Kesterson.

The similarly elevated blood Se concentrations in Kesterson and off-site kestrels was not surprising, given the close proximity of two of the off-site boxes to the Grasslands National Wildlife Refuge. This refuge is known to have relatively high environmental levels of Se, due to releases of agricultural drainage water onto the refuge. It is likely that one or both members of these two off-site pairs foraged at least partially on the refuge. In future seasons, efforts will be made to locate nest boxes in uncontaminated areas near Kesterson, in order to provide more useful reference data. On average, blood Se concentrations in both groups of nesting birds were similar to the overall mean for nonbreeding kestrels trapped from 1994-1997 on Kesterson (6.4 ppm, dry weight). These concentrations are below that observed in kestrels fed a dietary Lowest Observable Adverse Effect Level (LOAEL) of 12 ppm Se, and are not expected to be associated with adverse effects in wild kestrels (CH2M HILL, 1997a). Compared with other predatory bird species sampled at Kesterson, kestrels tend to be similar in selenium accumulation to red-tailed hawks, but lower than barn owls, northern harriers, and loggerhead shrikes. These species differences are probably in large part due to differences both in relative amount of time spent foraging at Kesterson and on prey species selection.

Selenium concentrations in eggs of kestrels at Kesterson were less than blood Se concentrations of parent birds; this was unexpected, based on data from captive kestrels indicating an approximate blood to egg transfer factor range of 1.7-2.8. Given the variability inherent in the blood Se concentrations of wild birds, it is unlikely that this relationship can be verified in the field with opportunistic, single blood samples (from parent birds) that are

not closely timed with egg-laying. Compared with other bird species sampled at Kesterson in past years, kestrels in 1997 exhibited relatively lower selenium accumulation in eggs (mean for all bird eggs sampled 1989-1995 = 6.3 ppm selenium, dry weight). For the barn owl nest box, ratio of blood to egg was similar (about 2) to the expected ratio for kestrels.

Based on pellet and nest box content analyses, the dietary compositions of barn owls and kestrels from Kesterson are fairly typical for these species (Knight and Jackman, 1984; Marti, 1987; Yalden and Morris, 1990; Balgooyen, 1976; Rudolph, 1980; Collopy and Koplin, 1983). Invertebrates, which have previously been found to predominate in breeding kestrel diets in California, contribute almost twice as much Se to the kestrel diet as do vertebrate dietary items. Assuming an approximate 1:1 relationship between blood and dietary selenium concentrations (determined in captive kestrel studies; CH2M HILL, 1997a), and about 60% foraging on-site (indicated by preliminary telemetry data this year), the estimated dietary concentration of 8 ppm Se for kestrels breeding at Kesterson would predict about 4.8 ppm in the blood. That this predicted concentration is equivalent to the mean concentration of selenium in the blood measured in these kestrels indicates that the captive kestrel model of selenium accumulation may be useful for predicting wild bird exposures.

If the 1:1 ratio (diet:blood) is also assumed for barn owls, the close agreement between the blood (3.8 ppm) and diet (4.4 ppm) selenium concentrations of the single female barn owl sampled suggest that this individual forages primarily on the reservoir, in spite of the nest being located on the reservoir edge. This bird had previously been trapped on Kesterson and banded on July 4, 1995, north of the compound nest location. At that time, the Se concentration in her blood was 5.1 ppm. In 1996, Se concentrations were the same in both this adult and one of her chicks (4.9 ppm). Blood Se levels across years in this individual suggests stability of both foraging range and Se concentrations in dietary items. The latter has been observed during the course of biological monitoring of small mammals over this period at Kesterson.

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Section 6
Bird Population Surveys

Introduction

Bird surveys are an ongoing monitoring effort that provides information on changes in bird populations by monitoring population trends, changes in species use, and seasonal changes in bird abundance and diversity. These surveys have been conducted since 1988. This information helps to time and interpret other studies (i.e., wild bird sampling and bird nesting and reproduction studies) and allows month-to-month, season-to-season, or year-to-year comparisons of bird use of Kesterson.

Objectives

The objectives of the surveys conducted at Kesterson were to determine seasonal and long term changes in the use of the site by birds and to estimate the number of birds using the Kesterson per month on a daily use basis.

Methods

The bird surveys at Kesterson are conducted using two methods. Reclamation biologists estimated "daily averages" and "use days" for waterfowl, raptors, shorebirds, and some songbird species including blackbirds and swallows until December 1996. CH2M HILL biologists continued these surveys beginning in March 1997. Transect counts (Dawson, 1981) are used to count more secretive species and those that do not congregate in large flocks. These surveys are used to estimate long-term changes in bird use for species such as western meadowlarks, horned larks, sparrows, and other upland birds using Kesterson. Twice-monthly bird censuses from 1989 to 1993 have shown two peaks of bird activity at Kesterson: one in March prior to the reproductive season in early spring, and a second peak in the fall when migrating and wintering birds arrive.

The daily use surveys are conducted at least four times monthly by driving along the access roads within Kesterson, counting birds observed, and calculating the average monthly use for each species counted. Transect surveys are particularly suitable in extensive, open, uniform, or species-poor habitats (Bibby et al., 1992). Transect surveys use a walking census of premarked belt (strip) transects to determine bird species occurrence and species density twice during the year; birds are censused over a 3-day period in early spring and again in the fall. The timing of the census depends on weather, daily bird use survey information, and other observations. Fixed transect belts (122 meters wide, 61 meters from center) were marked in 1989 and are used to help decrease variability by the observer by standardizing the observation area. These belts used for the 1989 through 1993 censuses were also used for the 1996 and 1997 censuses. The transect in Trisection 1 is 2.32 km long, the transect in Trisection 2 is about 4.05 km long, and the transect in Trisection 3 is 2.72 km long. To reduce the variation of estimates of bird numbers, belts have been subdivided into 50-meter intervals, further delineating the habitats. All birds seen or heard within the transect belts were counted, identified, and recorded on preformatted data sheets that included the following data: date, time census began and ended, transect identification, interval identification (type of habitat), species observed, and the number of individuals of each species seen or heard. Observations commenced either at sunrise and ended 3 to 4 hours later or began about 4 hours before sunset and ended by sunset. The direction and order that the transects are walked varied within the survey periods.

Species density was calculated by: $D = n/LW$; (D = density, n = number of observations of a species; L = transect (interval) length; W = transect width) (Emlen, 1988). Relative abundance was calculated by using the proportion of total birds of each species compared to the number of all birds of all species observed (Call, 1982; Dawson, 1981). Seasonal population estimates were calculated by multiplying the estimated density per acre of the species in the transect by the number of acres of the habitat type used by the species in the transect's corresponding trisection.

Results and Discussion

Bird use varies from year-to-year, probably in response to many factors such as habitat changes, food availability, and availability of cover. Many of the changes in bird species using Kesterson is partially due to the change in vegetative structure. The unvegetated and sparsely vegetated areas have become areas of tall, weedy vegetation. In terms of vegetation structure, as the vegetation at Kesterson moves through successional stages, the structure (i.e., dominant plant species height and density) changes and bird use within the habitat type changes. The distinction between the Open and Filled habitats is decreasing over time, although vegetation is generally less abundant in Open habitats. Birds such as sparrows that forage in tall weeds and grasses are as likely to be found in Open habitats as in Filled habitat. The low, dense perennial grasses in the Grassland habitats at Kesterson appear to support few seed- and insect-eating birds, possibly because food items are more difficult to find or less abundant than in the other habitat types.

Many of the birds using Kesterson, such as swallows, horned larks, meadowlarks, and killdeer, usually favor foraging and nesting in open, grassy habitat. Also, many of the raptors (e.g., red-tailed hawks and American kestrels) require open areas with perches for hunting. The Grassland habitats continued to show less growth of new vegetation and the thatch layer is thick in the Grassland areas (in some places about 0.3 meter thick), possibly discouraging foraging in this habitat.

Predatory Birds

Predatory birds (hawks, kites, falcons, and shrikes) are territorial during the breeding season and many maintain home ranges during the winter. Predatory birds were observed hunting and roosting at Kesterson and in adjacent areas throughout the year. The number of raptors (hawks, kites, and falcons) using Kesterson generally increases at the end of the breeding season (June) when young are dispersing from their natal areas and territoriality is reduced. Numbers remain high until spring (March) when only resident birds remain and set up breeding territories (Table 4 - 1). In 1994, there were significantly more juvenile red-tailed hawks captured during banding studies than in subsequent years and both red-tailed hawks

and American kestrels were observed in greater numbers wintering at Kesterson during surveys that year. Based on trapping results from 1994 to 1997, the number of barn owls was also significantly higher in 1994 than in other years.

Table 6-1. Daily Use of Kesterson by Raptors[1], 1989 to 1997

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1989	9.83	20.00	--	--	3.44	4.39	5.93	7.35	10.06	12.42	22.12	26.43
1990	25.25	24.00	27.00	14.62	17.64	13.62	12.61	13.28	15.50	--	27.27	37.73
1991	37.08	38.90	27.00	22.50	14.00	11.50	33.00	11.00	--	--	--	--
1992	--	--	10.25	6.00	6.36	7.25	5.75	11.50	6.18	10.86	15.64	27.00
1993	21.64	25.36	23.45	11.90	10.13	10.28	14.40	14.10	13.93	21.85	29.57	34.07
1994	36.21	31.50	21.86	11.13	12.93	13.07	32.00	26.63	25.27	30.67	35.75	41.87
1995	34.79	36.87	25.00	16.80	11.00	12.64	21.56	16.09	15.09	21.18	29.45	28.08
1996	27.45	21.27	17.50	9.64	8.88	12.20	20.30	15.47	13.44	--	--	--
1997	--	--	12.34	7.40	8.76	9.71	8.47	12.75	9.50	28.25	41.75	--
Average	**27.46**	**28.27**	**20.55**	**12.5**	**10.35**	**10.52**	**17.11**	**14.24**	**13.62**	**20.87**	**28.79**	**32.53**

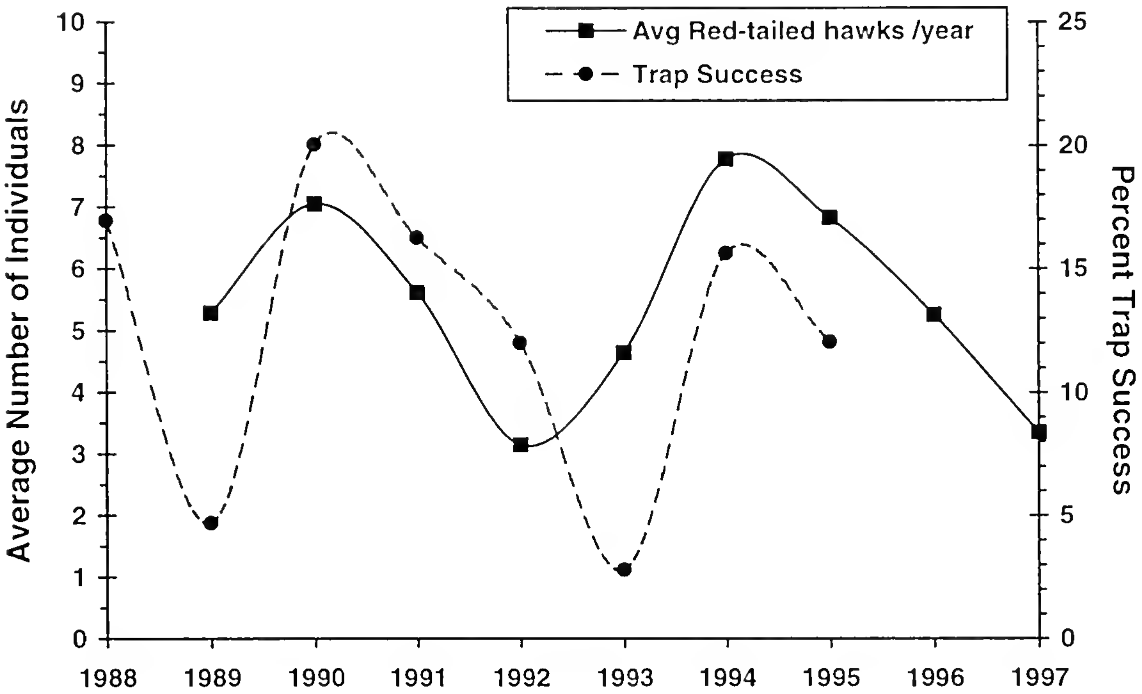
[1] The number of raptors using Kesterson per day averaged for each month

Two of the most commonly observed species at Kesterson with different hunting strategies and nesting requirements are the northern harrier and red-tailed hawk. Harriers, unlike many hawks, forage in fairly dense vegetation. Reported territories defended by harriers range from 0.8 to 110 ha during the breeding season and 3.9 to 124.9 ha (mean 33.6 ha) during the winter (MacWhirter and Bildstein, 1996). Analyses of raptor pellets (mostly northern harrier) collected from below feeding perches on Kesterson show that California voles are the most common small mammal preyed on, followed by pocket gophers. Kangaroo rats, house mice, deer mice, harvest mice, birds, and grasshoppers were also found in pellets but made up a smaller proportion of their diets. Red-tailed hawks forage most successfully by perching on a high point, such as a tree or power pole, surveying the area for prey, and dropping down

on their prey when it is sighted (Johnson, 1981; Preston and Beane, 1993). They have large home ranges (from 31 to 344 ha [Preston and Beane, 1993]) with open areas for foraging and are probably not foraging on-site to the same extent as the harriers. The power poles throughout Kesterson are a major attraction to raptors, but the high prey densities in or near open, sparsely vegetated patches on-site may also attract them.

Small mammal (i.e., deer mice, western harvest mice, house mice, California voles, and ornate shrews) abundance and availability (as prey) can be approximated by trapping success. From 1988 to 1995, small mammal trapping has been conducted at Kesterson from February through June. Although other factors affect the raptor populations at Kesterson, raptor numbers (especially red-tailed hawk) generally follow the prey base as estimated by the percent trapping success (Figure 6 - 1). Small mammal trap success and red-tailed hawk daily use declined from 1990 to 1992, increased in 1994 and was again lower in 1995. Based on the correlation between trap success and daily use, it appears that small mammal numbers have declined at Kesterson in 1996 and 1997.

Figure 6-1. Red-tailed Hawk Average Annual Daily Use and Small Mammal Trapping Success, 1988 -1997



White-tailed kites forage almost exclusively on small mammals (Dunk, 1995). The number of white-tailed kites observed using Kesterson typically is highest in October through November. During this period in 1997, the number of kites observed was 56 percent (October) and 66 percent (November) lower than in those same months from 1993 to 1995. However, during this same time period, northern harrier numbers have increased two to three fold.

Since the nest boxes were set up at Kesterson in 1996, kestrel numbers have been increasing. The nest boxes provide night roosts, hunting perches, feeding perches, and nest sites for kestrels and other birds. As kestrel pairs occupy nest boxes over time, it is expected that the population of resident kestrels that overwinter at Kesterson will increase. It is not yet known if the increase in kestrels will affect the population of loggerhead shrikes or other species by increasing competition and predation. Although male kestrels and shrikes utilize similar

habitat, shrikes and kestrels generally exhibit a high degree of habitat separation (Gawlik and Bildstein, 1995).

Loggerhead shrikes are predatory songbirds that eat invertebrates and small vertebrate prey. These birds show a relatively high breeding site fidelity (i.e., greater than 50 percent return to their breeding sites) and may maintain territories year-round, excluding all other shrikes (Yosef, 1996). Shrikes maintain relatively small territories (average 8.5 ha; Miller, 1931) ranging from 4.4 to 16 ha (Miller, 1931). At Kesterson, a small population ranging from about two to five pairs has been observed.

Songbirds

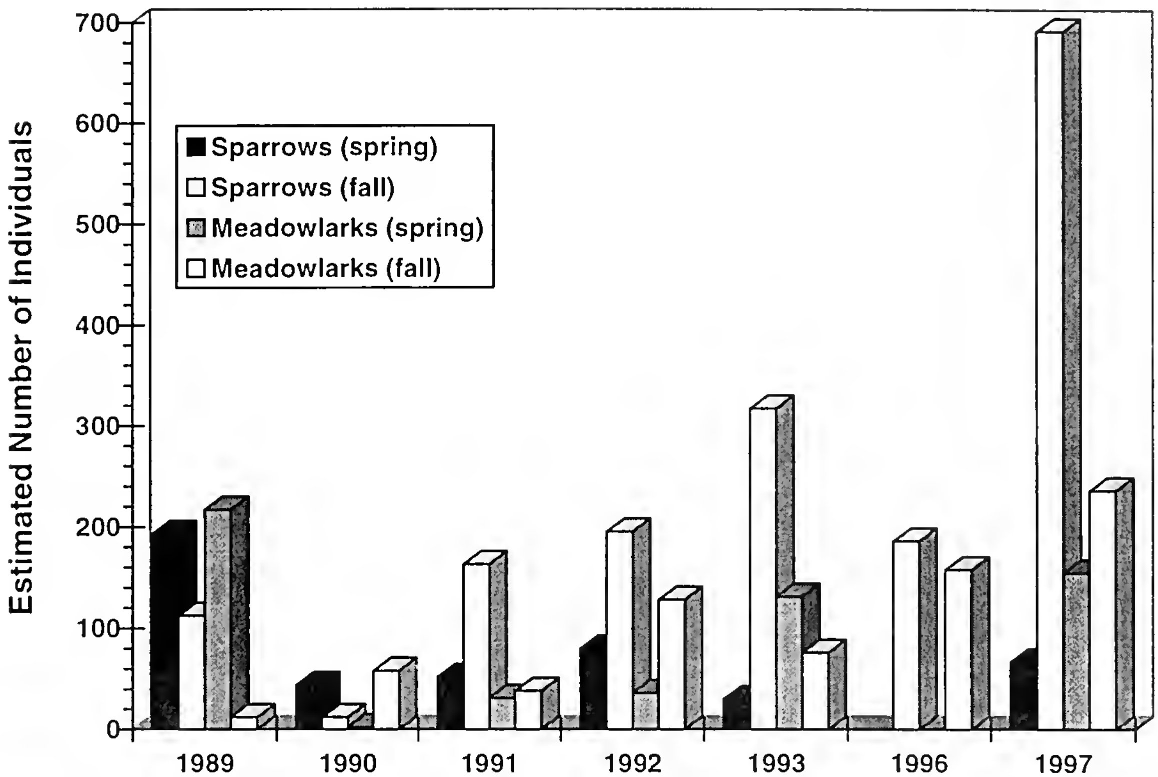
Savannah and white-crowned sparrows are the most abundant sparrows observed at Kesterson during the winter. Other sparrows typically observed in lower numbers are song sparrows and grasshopper sparrows. These birds feed almost exclusively on seeds during the winter (Chilton et al., 1995; Vickery, 1996; Wheelwright and Rising, 1993). Greater numbers of sparrows were observed during fall surveys when birds are wintering at Kesterson than were observed during spring surveys when most have left for their breeding grounds (Figure 6 - 2). This supports the findings of the nesting surveys that few species of birds nest at Kesterson (see Section 4). Sparrows observed during the fall surveys were associated mostly with dense cover and were observed foraging for seeds in the tall vegetation in all three habitat types.

The number of songbirds (sparrows and western meadowlarks are two of the most abundant species at Kesterson) using Kesterson has varied from year-to-year since 1989 (Figure 6- 2). Typically, greater numbers of songbirds are observed at Kesterson in fall, although this depends on a number of variables probably including climate, food availability (production), and reproductive success of birds in their breeding grounds. The estimated number of sparrows using Kesterson in the spring has dropped since 1989 and the estimated number observed in the fall has increased after initially dropping in 1990 (Figure 6 - 2). Although sparrow numbers were lower in fall 1996 than in fall 1993, generally the number of sparrows and meadowlarks estimated to be using Kesterson in the fall has increased during the survey

period. The increased numbers may be due, in part, to the change in conditions since the end of the drought in 1994.

Western meadowlarks continue to be abundant in spring and fall surveys. However, their greater abundance in spring, based on lack of nests found during nest surveys, is due to their foraging at Kesterson. Kesterson provides grassland habitat which meadowlarks prefer for foraging on (primarily) invertebrates in the spring. However, the thick layer of thatch found in the Grassland habitat makes it unsuitable for nesting. The number of western meadowlarks observed at Kesterson in spring surveys in all years since 1989 was lower than that observed in 1989 although increased numbers were counted in 1993 and 1997. The number of meadowlarks observed in the fall increased in 1992, 1996, and 1997 over other years surveyed.

Figure 6-2. Summary of Spring and Fall Estimates of Sparrows[a] and Meadowlarks



[a]Sparrows include: grasshopper, white-crowned, savannah, and song sparrows.

Note: Spring surveys are averages of April and May surveys and fall surveys are averages of November and December surveys. 1997 fall surveys were conducted in October.

Swallows are aerial insectivores that prefer open areas for foraging where they capture flying insects over vegetation throughout Kesterson and in adjacent areas. The number of swallows at Kesterson varies from year-to year (Table 6 - 2) but generally shows two peaks: one during early spring (March and April) and a second from late summer to early fall (September and October). These peaks coincide with spring and fall migration for these species and are made up of mostly tree swallows and cliff swallows. Relatively few swallows nest at Kesterson, presumably because of the lack of nesting sites.

Table 6-2. Daily Use of Kesterson By Swallows[1], 1989 to 1997

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1989	0.00	0.00	--	--	68.22	70.00	42.29	38.29	11.00	341.11	50.29	1.93
1990	20.08	19.89	380.67	186.24	127.79	33.31	44.06	69.89	15.00	--	24.67	3.47
1991	6.08	0.50	640.59	95.56	64.56	70.30	28.92	24.22	--	--	--	--
1992	--	--	426.00	104.50	66.36	181.13	47.88	13.50	6.55	1.43	33.43	17.38
1993	4.55	5.64	121.73	133.60	42.75	82.44	52.93	30.70	40.86	242.54	107.14	4.64
1994	11.50	28.50	163.07	166.50	79.64	54.07	22.00	20.06	171.00	213.92	32.38	0.47
1995	0.21	4.40	59.40	56.40	50.75	81.45	28.78	31.91	288.64	473.73	97.91	0.67
1996	4.73	33.82	82.83	190.00	46.25	73.00	11.00	32.47	31.89	--	--	--
1997	--	--	90.50	121.03	47.46	78.35	27.75	28.50	6.00	144.75	6.50	--
Average	**6.74**	**13.25**	**245.6**	**131.73**	**65.98**	**80.45**	**33.96**	**32.17**	**71.37**	**236.25**	**50.33**	**4.76**

[1] The number of swallows using Kesterson per day averaged for each month

Blackbirds (i.e., primarily red-winged blackbirds) continue to forage at Kesterson in high numbers from February through April and reach a low in August and September (Table 6 - 3). Blackbirds feed primarily on plant matter for most of the year and forage more on invertebrates during the breeding season (Yasukawa and Searcy, 1995). The number of blackbirds observed on Kesterson is typically highest in March and declines at the beginning of the breeding season for blackbirds (April) and continues to decline and remain low through the summer.

Table 6-3. Daily Use of Kesterson by Blackbirds[1], 1989 to 1997

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1989	0.00	0.29	--	--	18.9	7.3	0.57	0.00	3.3	31.7	45.7	77.5
1990	28.0	12.8	154.5	72.9	27.4	56.2	9.9	0.22	13.3	--	1.1	0.00
1991	0.08	1.8	42.8	31.3	34.2	68.5	7.0	7.3	--	--	--	--
1992	--	--	399.3	266.0	111.4	124.4	89.9	2.5	2.9	18.3	102.5	184.7
1993	132.3	97.1	303.7	225.5	85.3	58.0	95.1	56.3	18.9	20.1	27.0	13.0
1994	82.3	60.2	133.7	108.4	171.2	143.9	37.9	7.3	12.0	25.3	14.4	42.3
1995	107.9	512.1	549.4	237.6	118.6	60.9	36.7	5.3	2.4	4.2	8.3	69.7
1996	257.4	159.5	155.5	129.2	60.0	26.4	15.3	0.07	1.2	--	--	--
1997	--	--	311.2	118.0	54.4	32.4	9.2	1.8	16.5	74.8	94.5	--
Average	**86.9**	**120.5**	**256.35**	**148.6**	**75.7**	**64.23**	**33.5**	**8.97**	**8.8**	**29.0**	**41.9**	**64.5**

[1] The number of blackbirds using Kesterson per day averaged for each month

Shorebirds and Waterfowl

From 1989 through 1997, killdeer have accounted for 13 to 95 percent of the shorebirds observed at Kesterson. In 1997, killdeer accounted for 74 percent of the shorebirds counted. This is especially true during the reproductive period (February through June) during which the number of killdeer observed increased about 24 percent in 1997. Areas on Kesterson for killdeer to forage are limited, and they are mostly observed feeding along the drain that runs adjacent to the east side or along Mud Slough. In 1997, more areas of low-growing vegetation within Kesterson were found and greater numbers of killdeer were observed foraging and nesting in the interior and west side of Kesterson. In 1997, the highest concentration of shorebirds (not including killdeer) was observed in March (Table 6 - 4). The relatively high number of shorebirds seen in early spring at Kesterson coincides with availability of ephemeral rainwater pools which are greatly reduced by the end of March. This number abruptly drops in May as the nesting season progresses. This indicates that the

shorebirds only use Kesterson for resting and foraging and move to other locations for nesting.

Table 6-4. Daily Use of Kesterson by Shorebirds[1] (killdeer excluded), 1989 to 1997

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1989	0.25	0.14	--	--	0.00	0.00	0.00	0.12	0.00	0.00	1.35	4.57
1990	0.08	0.00	0.00	0.10	0.14	7.08	14.06	5.89	0.00	--	0.07	0.00
1991	0.00	0.00	0.18	0.25	0.00	95.20	19.67	0.11	--	--	--	--
1992	--	--	47.75	16.50	0.00	4.63	0.50	0.00	0.00	0.00	0.07	0.00
1993	0.18	1.82	10.73	92.70	1.00	6.00	6.07	0.40	0.00	0.08	0.14	0.29
1994	0.50	0.07	0.07	0.00	0.00	0.57	0.69	0.00	0.00	0.00	0.00	0.00
1995	0.07	3.80	11.53	16.00	2.17	3.91	0.78	0.00	0.00	0.00	2.73	1.42
1996	0.00	0.00	5.17	2.64	0.00	2.50	0.00	0.00	0.00	--	--	--
1997	--	--	21.17	2.80	0.00	0.00	3.17	0.00	0.00	0.00	0.00	--
Average	**0.15**	**0.83**	**12.08**	**16.37**	**0.37**	**13.32**	**4.99**	**0.72**	**0.00**	**0.00**	**0.62**	**1.05**

[1] The number of shorebirds using Kesterson per day averaged for each month

Waterfowl use has remained low since 1989 (Table 6 - 5), after Kesterson was dewatered and filled. The highest numbers of waterfowl observed are in the early spring when there are usually ephemeral rainwater pools on Kesterson that ducks utilize. Overall, waterfowl use was lower in 1997 than in other years surveyed. Duck abundance reached its highest levels in March and decreased in April and May.

Table 6-5. Daily Use of Kesterson by Waterfowl[1], 1989 to 1997

Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1989	0.00	0.00	--	--	7.33	2.50	0.57	0.00	0.00	0.00	0.00	0.00
1990	0.00	0.53	3.62	7.76	10.43	1.23	0.06	0.00	0.00	--	0.00	0.00
1991	0.00	0.00	2.41	2.25	1.39	0.20	0.00	0.00	--	--	--	--
1992	--	--	9.75	13.75	5.64	0.75	0.31	0.00	0.00	0.00	0.00	0.00
1993	0.00	5.64	14.55	14.80	6.63	2.06	0.60	0.00	0.00	0.00	0.00	0.00
1994	0.14	0.00	2.00	2.25	2.21	6.57	8.46	0.19	0.00	0.00	0.00	0.07
1995	0.43	4.87	21.07	23.00	9.83	9.82	0.44	0.00	0.00	0.00	0.00	0.00
1996	0.00	7.45	15.00	4.91	5.63	2.60	7.00	0.00	0.00	--	--	--
1997	--	--	9.83	2.60	1.00	0.14	0.00	0.00	0.00	0.00	0.00	--
Average	**0.08**	**2.64**	**9.78**	**8.91**	**5.57**	**2.87**	**1.94**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**

[1] The number of waterfowl using Kesterson per day averaged for each month

Most of the waterfowl and shorebird foraging and nesting habitat has been eliminated at Kesterson and these shorebird and waterfowl species were observed only occasionally in 1997. The avocets, stilts, and especially some species of ducks are birds that could potentially nest within Kesterson. Since filling of Kesterson, persistent rainwater pools that have formed have been filled so there have been no large pools that have persisted through the breeding season. Observations indicate that the numbers of birds foraging in the KNWR wetlands east of the San Luis Drain were typically greater than those observed within Kesterson and as the ephemeral pools recede, aquatic birds move off of Kesterson and onto the refuge and gun clubs in the area.

The tall vegetation in the Filled and Open habitats and the heavy thatch in the Grassland habitats will probably continue to discourage many birds from foraging and especially from nesting on-site. As succession of the vegetation continues, many of the annual plants now growing at Kesterson are being replaced by perennial plant species, such as perennial grasses

and shrubs, and bird density and species composition is also changing. The relevance of plant community structure to bird diversity is well documented (Karr, 1968; MacArthur and MacArthur, 1961). Many of the currently dominant tall plants such as five-hooked bassia and mustard are early invader species that are losing their dominance as less ruderal species begin to establish themselves, changing the vegetation structure at Kesterson and, therefore, changing the bird communities associated with it.

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Section 7
Kestrel Embryo Development

Introduction

Currently, the only species for which a complete and detailed description of development exists is the domestic chicken (Hamilton, 1952). Less detailed descriptions can be found for other precocial birds such as pheasants (Hermes and Woodard, 1987; Labisky and Opsahl, 1958), mallard (Caldwell and Snart, 1974), bobwhite quail (Roseberry and Klimstra, 1965), and Adelie penguin (Herbert, 1967). A description for American kestrel embryo growth by Bird et al. (1984) provides cursory photographic descriptions at three day intervals that is inadequate for use as a guide due to lack of detail and the quality of the photographic reproductions. Hence, while gross or extreme deformities are generally distinguishable in other bird species, more subtle morphological changes may be missed in the absence of a normal standard for comparison. Such errors in diagnosis can lead to inaccuracies in determining safe levels of exposure to a toxicant. In captive kestrel studies, kestrel embryos have been assessed using chicken developmental stages. Consistent morphological differences observed between kestrel and chicken embryos suggest that the chicken may not serve as an adequate 'baseline' for the kestrel. For the purpose of improving the assessment of embryo abnormalities in both captive and wild kestrels exposed to Se, a study was conducted to describe the normal development of kestrel embryos.

Objective

The objective of this study was to describe the normal embryonic development of the American kestrel in order to improve the assessment of embryos and offspring of captive and wild kestrels exposed to high dietary Se.

Preliminary Report

Captive kestrels maintained at the University of California at Davis were paired in mid-March for breeding. About forty pairs of kestrels were placed in individual breeding pens (6ft x 6ft x 5ft) and provided with nest boxes for breeding. Food, as commercial raptor chow supplemented with vitamins, and water were available at all times. Beginning two weeks after pairing birds, nest boxes were checked every-other-day. Any eggs found at the time of nest box checks were collected for artificial incubation. The daily development of incubating eggs was monitored via egg candling (non-invasive and nonlethal) and sacrificing embryos periodically during incubation. Photographs were taken of live embryos in the egg and of freshly sacrificed embryos and a photographic chart of normal kestrel embryo development is under development and will be submitted separately.

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Section 8
Sample Handling and Transportation/ Laboratory Analyses

Sample Handling and Transportation

Disposable plastic bags were used to transport materials to the CH2M HILL laboratory in Sacramento. The samples were sorted using stainless steel or Teflon forceps in glass or white enamel pans. All sorting equipment was rinsed with uncontaminated water after material from each sampling location had been processed.

After processing, each sample was placed in a chemically cleaned container (polyethylene bottle or whirl-pak) that was labeled in indelible ink with its unique code indicating taxon, collection date, pond number, habitat type, and sampling site number. This same information was also transcribed onto preformatted data sheets. The sealed, numbered samples were grouped by taxa and frozen immediately after sorting.

To avoid dead air space, the frozen samples were packaged in sturdy, insulated shipping boxes filled with dry ice. A chain of custody form accompanied the samples to the analytical laboratory. This form was signed and dated by both the sender and the recipient. The samples were shipped to the analytical laboratory by overnight delivery.

Laboratory Analyses at Laboratory and Environmental Testing, Inc. (LET)

Samples of both plant and animal tissue were analyzed for total Se content by hydride generation technique and atomic absorption spectrophotometry. Samples were prepared by lyophilization (freeze-drying), homogenization, and digestion. Lyophilization data were used to calculate percent moisture in all samples. Se detection limits were 0.1 to 0.3 ppm (dry

weight basis) in a sample of at least 0.5 g material. LET's quality control and quality assurance procedures include:

1. Duplicate analyses of 10 percent of samples
2. Analysis for recovery of spiked amounts in 10 percent of samples
3. Blank samples to assess contamination (1 with every 20 samples)
4. Blind reference standards in 5 percent of samples
5. Detailed tracking of all samples through the analysis steps

The mean and standard deviation of differences for the duplicate analyses were 104.7 +/- 2.5 percent, and 104.9 +/- 2.6 percent. These values indicate that the data can be used with a high degree of confidence in their accuracy. Samples collected from the site were analyzed by Environmental Trace Substances Research Center (ETSRC) from 1984 through mid 1992. In subsequent years, the samples were analyzed by LET using similar methods. Therefore, results are comparable among years.

Section 9

1998 Kesterson Reservoir Biological Monitoring Plan

Introduction

In 1987, a biological monitoring program for Kesterson Reservoir was implemented to document habitat and faunal changes and Se concentrations among selected plants and animals. Since then, biological monitoring has been conducted and annual reports have been presented describing these changes. Changes to the monitoring program were implemented based on a meeting of participants from U.S. Bureau of Reclamation (Reclamation), Regional Water Quality Control Board (RWQCB), U.S. Fish and Wildlife Service (Service), and CH2M HILL on September 11, 1995, and data collected during monitoring from 1987 to 1995.

An intermittent monitoring plan to meet the objectives set forth by the RWQCB for closure of Kesterson went into effect in 1996. Monitoring was augmented by several studies designed to evaluate the areas that pose the highest potential for Se risks to wildlife. The 1998 Biological Monitoring Plan for Kesterson Reservoir encompasses surveys, studies, and analyses to be conducted from December 1997 through November 1998. The elements of the biological monitoring plan are presented in Table 9-1.

The 1998 monitoring effort will reflect changes based on the results of the monitoring effort and studies conducted during 1996 and 1997. Field studies, surveys, and sampling will be conducted at Kesterson as indicated in Table 9-1. These studies will be discussed with the Service to refine the monitoring program for Kesterson.

Table 9-1. Intermittent Biological Monitoring and Focused Study Plan

Activity	1996	1997	1998	1999	2000	2001
Vegetation Surveys	-	-	X	-	-	X
Vegetation/Invertebrate/Soil Sampling	-	-	X	-	-	X
Small Mammal Sampling	-[a]	-	X	-	-	X
Nocturnal Surveys	-	-	X	-	-	X
Detritus and Mushroom Sampling	X	X	X	-	-	X
Bird Population Surveys	X	X	X	X	X	X
Bird Nesting and Reproduction	X	X	X	-	-	X
Ephemeral Pool Monitoring	X	X	X	X	X	X
Captive Kestrel Study	X	-[b]	-	-	-	-
Wild Bird Sampling[c]	X[d]	X	X	-[e]	-[e]	X
Nest Box and Telemetry[f] Study	X	X[f]	X[f]	-	-	X
Body Condition	X[d]	-	-	-	-	-

[a]Selected small mammals were collected for body condition studies.

[b]Kestrel embryo development study.

[c]Shrike sampling and banding study is included with wild bird sampling studies.

[d]Selected wild birds trapped were used for body condition studies.

[e]Only banding will be conducted.

[f]The telemetry portion of the study began during the second year of the study (1997).

Objectives

The objectives of the biological monitoring program are to maintain continuity of monitoring data and to comply with the State Water Resources Control Board Order No. WQ-88-7:

1. Assess the impact of Kesterson Reservoir on local and migratory wildlife.
2. Provide a basis for adjusting Kesterson Reservoir management.
3. Verify the effectiveness of cleanup actions at Kesterson Reservoir.
4. Provide a basis for modifying future biological monitoring.

The objectives and methods for the biological monitoring and focused studies that will be conducted in 1998 are presented below.

Field Sampling and Surveys

Vegetation Survey

Objectives

The purpose of the vegetation studies will be to assess recent changes in the plant species in terms of presence and cover. The data will allow a tracking of changes in wildlife habitat which can then be compared with wildlife use. Specifically, data collection from vegetation and physical environmental parameters can be analyzed to assess: 1) vegetation type changes in composition and structure; 2) how the different plant species and vegetation types, in the different habitat areas at Kesterson, are accumulating and processing Se; and 3) what projections for vegetational change may occur in the future and what vegetation and habitat management could be implemented to improve wildlife habitat and reduce Se toxicity concerns to wildlife species.

Methods

Vegetation Monitoring:

1) Plant species presence will be recorded and percent cover will be estimated. These data will be gathered within the eighty-seven permanent 1 m^2 plots established in 1991 within the belt transects used for wildlife censuses. Plant voucher specimens will be collected and retained for future reference and for any necessary confirmation of identification.

2) Field monitoring of vegetation will occur during March, May, and September. These months correspond with the optimal periods of the growing season at Kesterson during which all important plant phenological stages can be observed.

3) Soil samples will be collected at 45 of the 87 permanent plots. The 45 sample sites will be randomly selected with approximately equal numbers from each of the three vegetation types. Soil samples will be collected (in addition to or as part of the other soil-

sampling efforts that will be conducted) using a soil auger and taken to a depth of 15 cm. Soil analyses will include pH and salinity.

4) Soil moisture will be monitored at a representative site of each of the three vegetation types. Soil moisture will be measured through the vegetation monitoring period, March through September. Gypsum blocks will be buried into the soil and connected to a datalogger. The datalogger will take a soil moisture reading once per day at 6 A.M. during the monitoring period. Ten gypsum blocks will be positioned at each of the three stations. In addition, soil and air temperature, humidity and photosynthetically active radiation (PAR) will be recorded simultaneously.

Data Analyses

1) Vegetation field data will be entered into a computer database. Number of species encountered will be counted. Plant species cover values will be statistically analyzed for mean and standard deviation for all plots in each trisection, over all three monitoring periods, and within each vegetation type. Vegetation data will be analyzed using appropriate methods such as hierarchical cluster analysis of two-way indicator species analysis (TWINSPAN, Hill 1979) to determine if changes in plant species associations have taken place.

2) All soil data and other environmental data will be entered into a computer database. The data will be averaged and standard deviations calculated for all sample sites and environmental stations within each trisection and within each vegetation type.

3) Direct data analyses will be performed using scatter plots and line graphs for percent vegetation cover changes since the previous monitoring year (1995) for the three vegetation types, selected dominant plant species, in all trisections.

4) A multivariate analysis of plant species cover values for all plots against the soil and environmental data will be performed. The multivariate analysis will consist of performing a canonical community ordination by partial detrended canonical correlation, principal components analysis, and redundancy analysis (CANOCO, Ter Braak 1988). The analyses

will determine which plant species correspond optimally with particular soil and other environmental parameters.

Vegetation/Invertebrate/Soil Sampling and Analyses

Objectives

The primary objective is to determine the level of Se accumulated in abundant vegetation and invertebrates at Kesterson. Soil samples will be analyzed to determine the Se readily available to plants through the soil. The results of the analyses will be used to improve the predictions of how Se moves from soil to plants to animals.

Sampling Frequency

The peak period of potential exposure to Se for birds at Kesterson occurs during the reproductive (nesting) season from about the middle of February to the end of June and the overwintering period from the middle of December to the end of February. Samples will be collected once for each plant species or invertebrate type during this time period (February through July). The actual times when sampling occurs for each species will vary depending on environmental conditions that would affect the timing of plant and invertebrate occurrences and wildlife use of Kesterson (i.e., increased use due to changes in habitat, management, or flooding). This will result in continued sampling throughout the peak periods of potential wildlife exposure.

Sampling Locations

For sampling purposes, Kesterson has been divided into three trisections: The southern trisection (ponds 1, 2, 3, and 4), the central trisection (ponds 5, 6, 7, and 9), and the northern trisection (ponds 8, 10, 11, and 12). A minimum of six stations will be sampled in each macro habitat occurring in each trisection.

Where feasible, sampling locations will remain the same as locations sampled in the past. Sampling sites will be chosen by the biologists during peak wildlife activity and plant growth times.

Sampling Methods

Vegetation

Above-ground material will be clipped from living plants no less than 1.5 cm above the soil level. Samples will be collected from a sufficient number of plants (at least three) to provide at least 2 g wet weight each of vegetative material and placed in freezer bags and frozen. Plant species to be sampled are based on presampling reconnaissance and discussions with USFWS personnel, abundance and potential importance to wildlife, and predictions of future plant communities.

Invertebrates

Terrestrial invertebrates will be collected by pitfall traps, sweep nets, and hand collection. To collect ground-foraging insects, pitfall traps will be set at each sample site. Moist soil and grass will be placed at the bottom of the traps and a cover will be placed above the trap opening for protection from the elements. Sweep nets will be used to collect terrestrial insects that forage above the ground in the vegetation.

Active searching (e.g., turning over rocks, boards, and debris found in the trisections and plywood placed there) will also be used as a collection method. A sufficient number of invertebrates will be collected to provide at least 2 grams wet weight of each sample whenever possible. All invertebrates collected will be placed into labeled ziploc plastic bags or plastic jars and frozen. The samples will then be sorted and identified.

Soil

Soil samples will be collected at the end of the dry season (August or September) at each sample station where vegetation and invertebrates were collected. The soil will be collected by inserting a 4-cm corer for collecting 0-15 cm, 15-50 cm, and 50-100 cm soil samples.

Each sample will consist of a composite of three samples from the same depth collected within a radius of about 7.5 feet of the station center. The samples will be collected in cooperation with Lawrence Berkeley Laboratory (LBL) and shipped to LBL for analysis of the total water-extractable and total selenium. Detritus samples will be collected along with the soil samples by taking the layer of debris on the soil surface.

Small Mammal Collections and Analyses

Objectives

The objectives of this survey are to (1) document the levels of Se in small mammals at Kesterson, (2) determine the food items eaten by small mammals; and (3) monitor the reproductive condition of small mammals at Kesterson. Small mammals are the major prey base for higher trophic-level birds, reptiles, and mammals, including the San Joaquin kit fox (if present on site).

Methods

Trapping Methodology

Mammals will be trapped from February through June in each trisection. This time period coincides with the time period for mammal collections at Kesterson since 1987. Trapping locations will be chosen to correspond with invertebrate, vegetation, and soil sampling sites in each trisection. An effort will be made to collect target species from areas in which they were collected in previous years. Bird censuses and mammal trapping will overlap; however, setting and collecting traps will not be done while the census is being conducted. Museum Special snap-traps (14 x 7 x 0.6 cm) and Sherman live-traps will be baited with peanut butter and placed in suitable or available habitat at each location for trapping deer mice, western harvest mice, Heermann's kangaroo rats, and house mice. Ornate shrews and California voles will be analyzed for Se when at least three individuals are captured in a trisection. Trapping will continue until the target of 10 deer mice from each trisection is met or the end of June. All rodents of the other listed species (above) caught while trapping deer mice will be saved for possible analysis.

Analysis of Stomach Contents

The stomach contents of animals collected will be examined microscopically to identify plant or invertebrate fragments to determine their diet. Rodents chew vegetation very finely making identification by gross examination impractical; a staining procedure will be used for identifying plant material. Invertebrate will be identified by using a dissecting microscope to identify fragments and body parts. Material from the stomachs of animals collected will be stained and matched against a reference set for identification (Westoby et al., 1976; Williams, 1962).

Analysis of Reproductive Status and Measurements

Reproductive status of males will be determined by testes size and location. Status and productivity of females will be determined by mammary gland condition, implantation scars, as well as by the presence or absence of embryos and embryo size (Christian 1950; Conaway 1955). Weight and standard measurements will be made and animals will be frozen for possible future analyses.

Sample Preparation

Up to 10 individuals of each collected target species will be selected from each trisection for laboratory analysis to determine Se content.

Digestive tracts from each animal will be severed at the esophageal and pyloric sphincters. Stomachs will be opened lengthwise beginning at the pylorus, and the contents will be removed and saved for identification of food items. Each stomach will then be placed in chemically cleaned sample containers along with the animal from which it came. The liver will be removed from each carcass and will be analyzed for Se concentration. Whole body selenium levels for individuals will be calculated using the equation presented in Ohlendorf and Santolo (1994). Samples will be weighed, labeled, and refrozen for possible future analyses.

Nocturnal Surveys

Objectives

Nocturnal surveys will be conducted at Kesterson to determine use by nocturnal birds, mammals, and reptiles. These surveys may also allow us to determine whether Kesterson is used by the endangered San Joaquin kit fox which has been observed in the area in the past.

Methods

Nocturnal surveys will be conducted for three nights at least twice a month (weather permitting) during the fox denning season (March to June) and during the dispersal season (September to November) by conducting spotlight surveys and setting up bait, scent, and/or photo-monitoring stations at Kesterson. A record will be kept of all bird, mammal, and reptile observations made during these surveys. If kit fox use occurs at Kesterson, the frequency of surveys will be re-evaluated and increased if necessary.

Detritus and Mushroom Sampling

Monitoring is being conducted to characterize the exposure risks associated with detritus and mushrooms and to better characterize the detritus pathway. Organisms that feed primarily on detritus, such as sowbugs and mushrooms, have higher Se concentrations than herbivores, omnivores, and carnivores that have been analyzed. These detritivores are expected to continue to have elevated Se concentrations.

Methods

Detritus

Detritus samples will be collected at each of 54 sample stations by separating the organic layer of material from the mineral soils. Each sample will consist of a composite of three samples collected within a radius of about 24 ft (about 8 m) of the stations' center. Samples will be analyzed by Laboratory and Environmental Testing, Inc. (LET) for total Se.

Mushrooms

Mushroom samples will be collected from Kesterson in all seasons and locations (when and where available). They will be found opportunistically during other monitoring activities and by using systematic surveys of sparsely vegetated areas where mushrooms have been found in previous years. Samples for analysis will be collected by removing sections of the gill and cap from three or more fruiting bodies (when available). The cap and gill sections of the mushroom will be used because earlier studies showed that the highest Se levels were found in these parts of the fruiting body (USBR, 1992). The remainder of the fruiting body will be removed and frozen for future disposal to limit further propagation of mushrooms at Kesterson.

Bird Population Surveys

This is an ongoing monitoring effort that provides information on changes in bird populations by monitoring population trends, changes in species use, and seasonal changes in bird abundance and diversity. This information helps to time and interpret other studies (i.e., wild bird sampling and bird nesting and reproduction studies) and allows month-to-month, season-to-season, or year-to-year comparisons of bird use of Kesterson.

Methods

The bird survey will consist of two parts: 1) Continue estimating "daily averages" and "use days" for killdeer, raptors, blackbirds, swallows, and other selected bird species; 2) Transect counts will be used to estimate long-term changes in bird use for western meadowlarks, horned larks, sparrows, and other upland bird species at Kesterson. Bird censuses conducted since 1989 have shown two peaks of bird activity at Kesterson Reservoir, one in March during the reproductive season or spring migration (depending on species) and a smaller peak in the fall. Therefore, transect counts will be conducted during these seasonal peaks.

Long-term changes in bird numbers will be monitored using point or transect counts (Dawson, 1981). A walking census of premarked belt (strip) transects will be made to determine bird species occurrence and species density at least twice during the year. Birds

will be censused over a 3-day period in early spring and again in the fall. The timing of the census will depend on weather, daily bird use survey information and other observations. Fixed transect belts (122 meters wide, 61 meters from center) were marked in 1989 and will be used to help decrease variability among surveys. These belts used for the 1989 through 1997 censuses will be used for the 1998 censuses. The transect in Trisection 1 is 2.32 km long, the transect in Trisection 2 is about 4.05 km long, and the transect in Trisection 3 is 2.72 km long. To reduce the variation of estimates of bird numbers, belts have been subdivided into 50-meter intervals, further delineating the habitats. All birds seen or heard within the transect belts will be counted, identified, and recorded on preformatted data sheets including the following data: date, time census begins and ends, transect identification, interval identification (type of habitat), species observed, and the number of individuals of each species seen or heard. Observations will begin either at sunrise and end about 4 hours later, or about 4 hours before sunset and end at sunset. The direction and order that the transects are walked will be varied.

Density will be calculated by: $D = n/LW$; (D = density, n = number of observations; L=transect (interval) length; W = transect width) (Emlen, 1971). Relative abundance will be calculated by using the proportion of total birds of each species compared to the number of all birds of all species observed (Call 1982; Dawson, 1981). Monthly population estimates will be calculated by multiplying the estimated density per acre of the species in the transect by the number of acres of the habitat type used by the species in the transect's corresponding trisection.

Bird Nesting and Reproduction

The objectives of this study are to determine Se concentrations in eggs as well as to assess the reproductive success of birds nesting at Kesterson Reservoir. Reproductive success will be evaluated by determining the frequencies of embryonic mortality and developmental abnormalities, and the hatching and fledging success of birds nesting during spring and summer of 1998.

Nest Searches

From mid-February through June 1998, biologists will search for and monitor nests of water-related birds such as shorebirds and waterfowl (especially if ephemeral pools are present), and terrestrial species such as lesser nighthawk, northern harrier, barn swallow, and meadowlark at Kesterson.

Nests will be located using a variety of techniques opportunistically during other activities, systematic searches in appropriate areas (e.g., grassland areas where waterfowl are observed), by observing nest defense behaviors (e.g., northern harriers stooping on observer close to their nest), and by searching known nest sites (e.g., barn swallow nest in culverts). The levee roads will be searched for killdeer, lesser nighthawk, and other nests. These birds are usually visible from the roads surrounding the ponds. Nests will be located by driving slowly along the levee roads and watching for the adult leaving the nest, displaying, or "sneaking" away.

Nest Monitoring

After a nest is found, it will be marked by placing flagging approximately 10 feet away from the nest (i.e., north and south of the nest), mapped, and given a unique code. Nest codes will include the initials of the biologist that found the nest, a four-letter species acronym, the pond number, and a nest number. Monitoring of the nest will begin immediately after its discovery and continue on a weekly basis to assess hatching success, chick survival, and fledging success. Monitoring will be conducted with minimum disturbance to the nesting birds. Each week the date, nest code, and number of eggs will be recorded on data sheets. We will also attempt to determine if the embryo is alive or dead and the approximate embryonic stage of development by candling the eggs with a modified flashlight or radiator hose.

Egg Collections

One egg will be removed immediately after a nest is located (only if the nest contains more than one egg). As soon after collection as possible, the egg will be opened to determine the developmental stage. The contents of the egg will be saved for Se analysis. Gross embryonic abnormalities are easiest to detect at late stages of development (ideally after limbs have

developed). If the embryo in the first egg removed from a nest is not sufficiently developed to detect abnormalities, a second egg may be removed at a later date. At collection, each egg will be marked with its nest code and the date it was removed. The data will be recorded on a data sheet and the egg will be placed in a container to avoid damage. All eggs removed from nests will either be examined or refrigerated within 1 hour of collection.

Laboratory Examinations

Each egg will be opened to determine its fertility, stage of development, viability, the position of the embryo, and whether pipping (internal pipping into the air cell or external pipping of the shell) has occurred. Each embryo will also be examined for evidence of external deformities. The entire egg contents will be saved in chemically cleaned containers and frozen until shipment to the laboratory.

Ephemeral Pool Monitoring

Rainwater pools that form at Kesterson over the winter months and persist for a month or more have been observed to develop aquatic invertebrate populations. Sampling in recent years has revealed that the invertebrates of these pools accumulate elevated levels of Se in their tissue that could be potentially toxic to shorebirds or waterfowl feeding in these locations during their nesting season. The shorebirds or other wildlife may be exposed directly by drinking the water or through diet but the diet is the primary exposure route. Continued monitoring of the Se concentrations in the water and invertebrate tissue of ephemeral, rainwater pools is a means of tracking potential food chain exposure at Kesterson Reservoir. Sediment Se concentrations have not proven to be a valuable measure of Se exposure and further sediment sampling from these pools is not necessary.

Methods

The larger and deeper rainwater pools at Kesterson Reservoir will be observed in conjunction with other monitoring activities for evidence of persistence of greater than one month in the late winter period. Persistent pools will then be sampled in the mid-March to mid-April (and longer if they persist) period for water and aquatic invertebrates. Water samples will be

collected as grab samples to be analyzed for total Se. Invertebrates will be collected by kicknet or small aquarium nets, sorted from debris, separated to species (or as close as practical) in plastic pans in the field, and hand picked by forceps into Whirlpak bags for shipment to QAL (CH2M HILL Quality Analytical Laboratory, Redding, CA). All water samples will be chilled in the field. Water samples will be sent to QAL chilled within one day. Invertebrates will be frozen within one day and sent to LET frozen.

Wild Bird Sampling

This sampling and banding effort using non-lethal sampling methods provides data on Se exposure levels in wild birds and data for evaluating the risk from Se to terrestrial birds at Kesterson. Other goals of this effort are to provide information on survival, site fidelity, and use patterns for wild birds utilizing Kesterson.

Methods

Birds including American kestrel, northern harrier, red-tailed hawk, barn owl, great horned owl, and loggerhead shrike will be captured using live trapping methods for raptors (Bub, 1991) throughout the year. Once captured, birds will be processed (i.e., measured, weighed, sampled [blood], and banded with a U.S. Fish and Wildlife Service band) and released. Loggerhead shrikes and some kestrels will be given a colored leg band for field identification. Blood samples of 0.8 percent or less of the birds' body weight will be collected from brachial or jugular veins by an experienced avian biologist. Blood samples will be placed in sterile tubes marked with species, date sampled, and the band number and kept cool until delivery to the laboratory for Se analysis. Samples held for more than one day will be stored frozen.

Nest Box and Telemetry Study

The objectives of this study are to characterize Se exposure in kestrels or other cavity-nesting terrestrial species breeding at Kesterson and determine relationships among foraging patterns (that is, proportion of foraging taking place at Kesterson), nest success and Se accumulation. Opportunistic monitoring of wild terrestrial birds at Kesterson has produced no evidence of adverse reproductive effects, in spite of relatively high Se concentrations in dietary items and other media. The degree to which nesting birds are exposed to and accumulate dietary Se at Kesterson remains poorly understood, but is critical to assessing the potential for reproductive effects in these species.

Methods

Monitoring of nest boxes placed on Kesterson in 1996 was conducted during the 1996 and 1997 breeding seasons and will again be conducted begining in mid-February of 1998. Proximity to and entry into or exiting of nest boxes by pairs will be observed with binoculars at a non-disruptive distance to determine nest box occupancy. Occupied nest boxes will be checked weekly for eggs and chicks. One kestrel egg will be collected from each box for Se analysis. All starling eggs will be collected from each box they are found in and an egg will be randomly sampled from each clutch for Se analysis. In addition, blood samples from adult kestrel males and females will be collected whenever possible. Females kestrels will be captured at night in the nest box and males will be trapped using live bait (bal-chatri trap, Bub 1991). Blood and liver samples will be collected from starlings found in nest boxes. Male and female kestrels will be banded with USFWS bands and color-banded for individual identification in the field. In addition, males will receive radiotransmitters and will be followed via radiotelemetry at least once a week throughout the breeding season (i.e., until fledging of young). Locations obtained will be used to assess foraging range and the extent to which foraging occurs at Kesterson. In instances where the male of a pair cannot be captured, the female will be radiotagged and tracked when she begins foraging to feed chicks. Clutch size, hatchability of eggs, incidence of abnormalities, and chick survival to fledging will be determined for each nest. Regurgitated pellets will be collected in and below the nest box and analyzed to determine type and number of dietary items and to facilitate dietary Se

estimations. Kestrel chicks will be examined for health and development, banded and blood sampled (for Se analysis) at two to three weeks of age.

Bird Collections and Analyses

Background

Killdeer and meadowlarks are common bird species found at Kesterson. Se concentrations (liver and blood) and dietary items have not been characterized in killdeer using Kesterson, and meadowlarks have not been sampled since 1990. Liver and blood are good indicators of recent dietary exposure when eggs can not be sampled. Se exposure has been primarily determined by collecting liver and/or eggs. Eggs are considered the most sensitive indicator of exposure for evaluating potential effects but can only be collected for a short time period and may be difficult to collect for species such as the meadowlark. Liver is a poor Se indicator for monitoring because it requires sacrificing the bird. Blood is a good indicator tissue for Se exposure because it reflects dietary exposure (CH2M HILL, 1997), can be collected during any season, and does not require sacrificing the bird so it has the potential for repeated sampling of individuals. However, most background information on Se in birds is from liver concentrations. Comparison of paired blood to liver Se to determine the usefulness of blood as a Se indicator has yet to be conducted in free-living birds. Food habits of killdeer foraging at Kesterson have not been determined. Some early-season killdeer eggs (February through April) have much higher Se than late-season (May through June) eggs possibly due to different location of nests (i.e., interior versus peripheral nests), time period (i.e., during the time when ephemeral pools are present on Kesterson), or availability of different food items.

Objectives

The objectives of this study are to 1) document the changes in Se levels over time in common birds at Kesterson, 2) determine exposure of killdeer and meadowlarks to Se, 3) determine the food items eaten by killdeer to characterize exposure pathways, 4) compare dietary items of meadowlarks to dietary items collected in other years (1988, 1989, and 1990) to identify

changes and to characterize the exposure pathway; and 5) compare Se concentrations in liver and blood to dietary items and other birds to characterize Se accumulation.

Methods

Collection Methodology

Killdeer and meadowlarks will be collected in February in each trisection. In this way, birds will be collected prior to the breeding season and no significant effects to overall reproduction for these species is expected to occur from this sampling. Killdeer and meadowlarks will again be collected in late June to compare seasonal differences. Birds will be collected by gun, and three or four birds will be collected from each trisection if possible (total of 10 to 12 individuals/species/period). Blood samples will be taken from each bird immediately after collection as an indicator of Se exposure and for comparison to liver Se. Digestive tracts from each animal will be severed and opened lengthwise, and the contents will be removed, preserved and frozen to stop digestion, and saved for identification of food items.

Food Item Analysis

The esophagus, proventriculus, and gizzard contents of birds collected will be examined microscopically to identify plant material (e.g., seeds, etc.) and invertebrate parts. The results will be compared to available published studies and Kesterson-specific data.

Sample Preparation and Analysis

Ten to 12 individuals of each collected target species from each trisection will be prepared for laboratory analysis to determine Se content. The liver will be removed from each carcass and will be analyzed for Se concentration. Livers and carcasses will be weighed, labeled, and frozen. Livers and blood will be sent to LET to be analyzed for total Se, which will be reported on a dry-weight basis.. Carcasses will be saved for possible future analyses.

Laboratory Studies

Captive Kestrel Studies

The Captive Kestrel Study, testing effects of Se on kestrel reproduction, has been completed and the results analyzed. Reports on those results have been submitted and have been submitted to scientific journals (Environmental Toxicology and Chemistry and Journal of Wildlife Management) for review and comments. Kestrels will be maintained during 1998 for possible future studies. If no additional studies are identified during 1998, the future of the colony will be evaluated and recommendations made in the 1998 Biological Monitoring Report.

Reporting

An annual report of the biological monitoring results will summarize the results of the current year's biological monitoring program. These results will be compared with results from biological monitoring conducted in the past at Kesterson Reservoir and with results from focused studies and other appropriate research programs. The results of ongoing and focused studies will be summarized in the annual report if the data are available. A detailed report on the results of each focused study will be prepared and submitted two months after the completion of the study or at a date agreed on by Reclamation and CH2M HILL.

The results of the biological monitoring program will be used to:

- Describe existing biological conditions at Kesterson Reservoir.
- Assess the impact of Kesterson Reservoir on the local biological community, including migratory birds.
- Describe an adjusted (if appropriate) biological monitoring plan or focused studies for the next year.
- Provide a basis for adjusting Kesterson Reservoir management, if appropriate.

- Provide information to further improve Kesterson Reservoir Risk Assessment model predictions.

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